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RESPIRATION OF CITRUS FRUITS AFTER HARVEST [1]

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INTRODUCTION

It is generally assumed that the rate at which a fruit respires after harvest is a measure of the rate at which it is deteriorating. If this assumption is correct, high respiratory activity should be associated with rapid deterioration or short storage life. Respiration is a chemical process, and, as with other chemical reactions, the rate increases greatly with temperature. On the other hand, the storage life of fruit does not necessarily decrease with increased temperature, as certain physiological disorders such as pitting of oranges, lemons, and grapefruit and membranous stain of lemons are associated with low temperatures, so that a longer commercial life of these fruits at the higher temperatures is associated with a more rapid rate of respiration. However, respiratory activity does indicate the rate at which the elaborated foods are being oxidized and the fruit is deteriorating in food value. This loss of food value may be extremely slow at low temperatures, but it assumes appreciable proportions at high temperatures.

The respiratory rate is also an indirect measure of the heat evolved by the oxidation of the carbon present, principally as sugar and acid in citrus fruits. This heat evolved is of particular interest from the standpoint of refrigeration of the fruit during transportation and storage.

A preliminary report on the relation of temperature to the respiration of certain fruits has been made (*6*); [2] this paper presents further data on the relation of temperature and other factors to the respiration of certain citrus fruits.

MATERIAL AND METHODS

The respiration determinations were made at the experimental cold-storage laboratory of the Bureau of Plant Industry, Soils, and Agricultural Engineering, near Washington, D. C., on oranges (*Citrus sinensis* (L.) Osbeck) and grapefruit (*C. paradisi* Macfad.) from Florida and California and on lemons (*C. limonia* Osbeck) from California. In the earlier experiments the Florida fruit was obtained on the Washington, D. C., market. In the later experiments the fruit was shipped to the experimental laboratory by unrefrigerated express directly from the point of production.

The respiration determinations were made by a method previously described (*7*), the apparatus being set up in the different constant-

[1] Received for publication November 23, 1943.

[2] Italic numbers in parentheses refer to Literature Cited, p. 358.

temperature rooms. By this method the amount of oxygen consumed, as well as the amount of carbon dioxide evolved, was measured. The determinations were usually run in duplicate at each temperature, and each run extended over a period of approximately 24 hours, except that at the low temperatures (32° and 40° F.) each run was generally of approximately 48 hours. (On the graphs such 2-day runs at the low temperatures are plotted as 1-day runs.)

At each temperature a period of 12 to 24 hours was allowed for the fruit to come to the temperature of the room before the determinations were started.

Results were tested for statistical significance by analysis of variance.

EFFECT OF TEMPERATURE AND VARIOUS OTHER FACTORS ON RESPIRATION

FRUIT TESTED

Florida Oranges

When the earlier tests (1929–30) were made on Florida oranges, facilities were not available to determine the respiration rate at all temperatures simultaneously; consequently, determinations were made on each lot at only a few of the temperatures. However, on each lot except No. 5 the rate was determined at 60° F., as a standard for comparison. The rates of evolution of carbon dioxide by the various lots at 60° did not differ significantly (table 1) except for one lot that was severely russeted; therefore, it seemed permissible to compare the averages for all temperatures, even though comparable fruit was not used throughout at all temperatures. The results are plotted in figure 1, *A*, for the carbon dioxide evolved, and in figure 2, *A*, for the respiratory ratio of the volume of carbon dioxide evolved to the volume of oxygen consumed. Generally runs were made at each temperature for at least 4 days, and the carbon dioxide determinations are plotted to show the change in rate with time. As will be seen (fig. 1, *A*), there was no appreciable change in rate with time at the lower temperatures (32°–50°). At 60° there was a slight increase and at the higher temperatures a decrease. The decrease was very marked at 90° and 100°.

Although four determinations were made on Washington Navel oranges at each of the temperatures, duplicate determinations at 32°F. were averaged; hence only two determinations are shown (fig. 1, *C*) for this temperature.

The curves show an increase in rate of respiration with each rise in temperature. At temperatures above 70° F. the carbon dioxide evolved generally increased to a greater extent than the oxygen consumed, with a consequent increase in the respiratory ratios (fig. 2, *A*). It is generally assumed that a ratio of 1.0 represents the complete oxidation of a hexose sugar to carbon dioxide and water and that a ratio of 1.33 represents the complete oxidation of citric acid. Intermediate ratios might represent the respiration of various proportions of sugar and acid (see table 10), whereas ratios above 1.33 probably are due to partial intramolecular respiration. Further consideration of the significance of the respiratory ratios will be found under Discussion (p. 353).

TABLE 1.—*Effect of temperature and other factors on respiratory activity of Florida oranges, 1929–30 and 1941–42*

[Respiratory activity expressed as milligrams of CO_2 per kilogram-hour]

Lot No.[1]	Date	Respiratory activity (CO_2 evolved)[2] at indicated temperature (° F.)								
		32	40	50	60	70	80	90	100	110
	1929	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	Dec. 3–7	-----	5.9	-----	20.4	-----	-----	-----	-----	-----
		-----	6.5	-----	21.0	-----	-----	-----	-----	-----
1	Dec. 12–14	3.0	-----	-----	21.9	-----	35.7	-----	-----	-----
		3.0	-----	-----	19.5	-----	35.0	-----	-----	-----
	Dec. 17–23	3.0	-----	-----	21.7	-----	-----	-----	-----	90.6
		3.1	-----	-----	20.5	-----	-----	-----	-----	-----
	1930									
2	Jan. 13–20	3.2	-----	-----	16.6	-----	-----	-----	70.3	106.1
		3.2	-----	-----	17.7	-----	-----	-----	75.7	106.5
	Jan. 20–25	-----	-----	-----	21.9	-----	-----	44.8	-----	-----
		-----	-----	-----	21.9	-----	-----	45.2	-----	-----
3	Jan. 27–Feb. 1	-----	-----	11.9	19.9	29.0	-----	58.2	-----	-----
		-----	-----	12.6	22.9	29.7	-----	42.1	-----	-----
	Feb. 3–8	-----	-----	-----	23.5	31.7	-----	44.8	-----	-----
		-----	-----	-----	-----	31.2	-----	44.0	-----	-----
4	Feb. 17–24	-----	6.1	-----	23.5	-----	-----	-----	-----	-----
		-----	7.1	-----	19.9	-----	-----	-----	-----	-----
5	Mar. 10–15	-----	-----	-----	-----	28.5	-----	-----	-----	-----
		-----	-----	-----	-----	29.3	-----	-----	-----	-----
6	Apr. 7–18	-----	-----	-----	18.6	-----	-----	-----	66.8	-----
		-----	-----	-----	20.2	-----	-----	-----	63.7	-----
Average, 1929–30		3.1	6.4	12.3	20.7	29.9	35.4	46.5	69.1	101.1
	1941									
7a	Nov. 28–Dec. 3	2.8	-----	17.9	-----	41.0	-----	-----	-----	-----
7b	do	3.0	-----	18.5	-----	38.0	-----	-----	-----	-----
8a	Dec. 6–12	2.1	-----	15.7	-----	30.8	-----	-----	-----	-----
		2.2	-----	14.9	-----	30.4	-----	-----	-----	-----
8b	do	2.0	-----	13.0	-----	26.6	-----	-----	-----	-----
		2.0	-----	12.5	-----	27.1	-----	-----	-----	-----
	1942									
9a	Mar. 24–Apr. 1	4.2	-----	19.9	-----	42.8	-----	-----	-----	-----
		4.7	-----	21.9	-----	40.1	-----	-----	-----	-----
9b	do	3.5	-----	20.5	-----	37.0	-----	-----	-----	-----
		3.5	-----	18.3	-----	37.2	-----	-----	-----	-----
10a	Apr. 28–May 6	2.9	-----	17.8	-----	37.3	-----	-----	-----	-----
		3.2	-----	17.2	-----	41.6	-----	-----	-----	-----
10b	do	2.6	-----	15.0	-----	33.2	-----	-----	-----	-----
		2.7	-----	15.3	-----	35.0	-----	-----	-----	-----
11a	June 2–8	2.5	-----	12.7	-----	27.4	-----	-----	-----	-----
		2.4	-----	12.1	-----	26.8	-----	-----	-----	-----
11b	do	2.2	-----	10.9	-----	24.5	-----	-----	-----	-----
		1.9	-----	11.5	-----	23.8	-----	-----	-----	-----
Average, 1941–42		2.8	-----	16.1	-----	34.0	-----	-----	-----	-----

[1] Lot 1, oranges purchased on Washington, D. C., market, Dec. 3, 1929; variety and previous history unknown. Lot 2, oranges purchased on Washington, D. C., market, Jan. 12, 1930; variety unknown; fruit heavily russeted. Lot 3, oranges shipped directly from Florida; received Jan. 18, 1930; variety unknown. Lot 4, oranges shipped directly from Nocatee, Fla.; received Feb. 15, 1930; variety unknown. Lot 5, Valencia oranges shipped Feb. 20, 1930, directly from Bradenton, Fla. Lot 6, oranges shipped Mar. 18, 1930, directly from Frostproof, Fla.; variety unknown. Lot 7, Washington Navel oranges on sour orange (*Citrus aurantium* L.) rootstock in heavy hammock soil, shipped directly from Citra, Fla., Nov. 24, 1941; received Nov. 27. Lot 7a, fruit from low ground; solids, 10.6 percent; acid, 0.62 percent; ratio, 17.1. Lot 7b, fruit from hilltop; solids, 10.7 percent; acid, 0.78 percent; ratio, 13.7. Lot 8, Parson Brown oranges grown on Norfolk fine sand, shipped directly from Orlando, Fla., Dec. 2, 1941; received Dec. 5. Lot 8a, sour orange rootstock; solids, 10.85 percent; acid, 1.42 percent; ratio, 7.6. Lot 8b, rough lemon (*C. limonia*) rootstock; solids, 9.9 percent; acid, 0.94 percent; ratio, 10.5. Lot 9, Valencia oranges (early picking) grown in Norfolk fine sand, shipped from Windermere, Fla., Mar. 9, 1942; received Mar. 12; held at 32° F. until beginning of experiment. Lot 9a; sour orange rootstock; solids, 13.2 percent; acid, 1.81 percent; ratio, 7.3. Lot 9b, rough lemon rootstock; solids, 11.3 percent; acid, 1.53 percent; ratio, 7.4. Lot 10a, same as lot 9a but picked and shipped Apr. 20, 1942; solids, 13.2 percent; acid, 1.42 percent; ratio, 9.3. Lot 10b, same as lot 9b but picked and shipped Apr. 20, 1942; solids, 12.1 percent; acid, 1.16 percent; ratio, 10.4. Lot 11a, same as lot 9a but picked and shipped May 29, 1942; solids, 13.4 percent; acid, 1.08 percent; ratio, 12.4. Lot 11b, same as lot 9b but picked and shipped May 29, 1942; solids, 11.0 percent; acid, 0.93 percent; ratio, 11.8.

[2] The 2 figures given for each date period represent determinations on duplicate samples of fruit.

The respiratory ratios for these Florida oranges (fig. 2, *A*) averaged about 1.0 at 32° F. and increased to about 1.20 at 50° to 70°. There was a further increase above 70° to a maximum of 2.0 at 100°. This would indicate that sugar was respired at 32° but that a higher per-

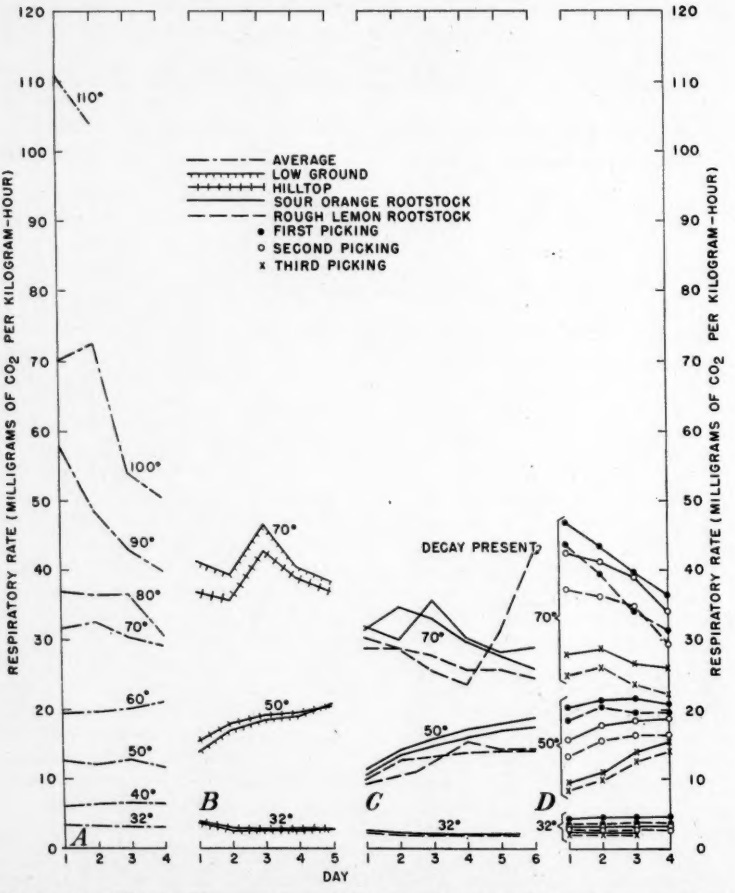


FIGURE 1.—Effect of temperature (°F.) on respiratory activity of oranges grown in Florida: *A*, Average for various lots, 1929–30 season; *B*, Washington Navel oranges from low-ground and hilltop soils, 1941 season; *C*, Parson Brown oranges from trees grown on sour orange and rough lemon rootstocks, 1941 season; *D*, three pickings of Valencia oranges from trees grown on sour orange and rough lemon rootstocks, 1942 season.

centage of acid than of sugar was respired at 50° to 70° or that possibly the sugar was incompletely oxidized, with the formation of intermediate products such as ethylene or alcohol. At 90° and above, the ratios indicated that considerable intramolecular respiration was taking place.

In the tests made during the season of 1941–42, only temperatures of 32°, 50°, and 70° F. were used and the influence of rootstock and maturity was investigated. There was a greater variation among

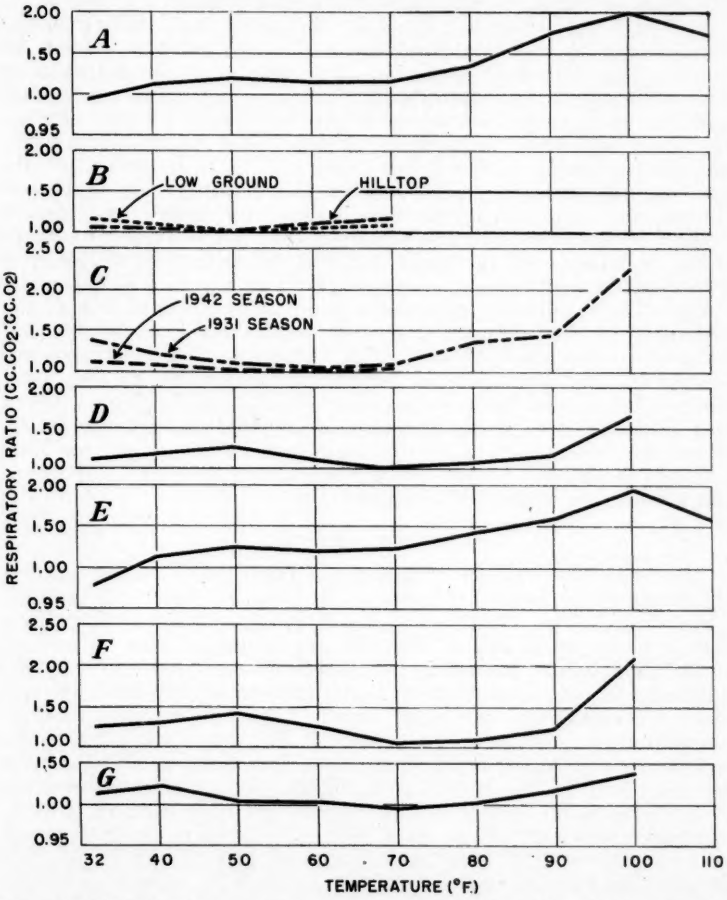


FIGURE 2.—Effect of temperature on respiratory ratio (cc. CO_2: cc. O_2) of Florida and California oranges, grapefruit, and lemons: *A*, Average for various lots of oranges grown in Florida, 1929–30 season; *B*, Washington Navel oranges grown in Florida, 1941 season; *C*, Washington Navel oranges grown in California; *D*, Valencia oranges grown in California, 1932 season; *E*, average for various lots of grapefruit grown in Florida, 1929–30 season; *F*, Marsh grapefruit grown in California; *G*, Eureka lemons grown in California.

these lots at a given temperature (table 1) than in the lots previously tested. Thus, at 32° the carbon dioxide evolved ranged from less than 2.0 to more than 4.0 mg. per kilogram-hour. The average of all lots at 32° for the season of 1941–42 was 2.8 mg. This was somewhat less than for the earlier season (1929–30). On the other hand,

the respiratory rate increased more with higher temperatures in 1941–42 than in 1929–30. The Washington Navel oranges (table 1, lot 7, and fig. 1, *B*) had fairly high respiratory activity at 50° and 70° with no consistent difference due to soil type.

Parson Brown oranges (table 1, lot 8, and fig. 1, *C*) were intermediate to low in respiratory activity. The rate averaged about 15 percent higher in fruit grown on sour orange rootstocks than in fruit grown on rough lemon rootstocks. The higher respiratory rate with the sour orange rootstock was associated with a higher concentration of both sugar and acid in the juice. The respiratory activity of Valencia oranges (table 1, lots 9–11, and fig. 1, *D*) varied greatly and was influenced by both rootstock and maturity. As in Parson Brown, the respiratory rate was consistently higher in Valencia oranges grown on sour orange rootstock than in those grown on rough lemon rootstock, and the sugar and acid concentrations of the juice were considerably higher in oranges grown on the sour orange rootstock. The respiratory rate was highest in the fruit picked at the beginning of the commercial harvest season (table 1, lot 9) and decreased in the later pickings. The average rate for the midseason picking (lot 10) was about 88 percent as high as that for the early picking (lot 9), and the average rate for the late picking (lot 11) was only about 62.5 percent as high as that for the early picking (lot 9). There was no reduction in the sugar concentration in the later pickings to account for the lower respiratory rates, although there was a reduction in the acid concentration.

California Oranges

The respiration rates of Washington Navel and Valencia oranges are presented in figure 3, *A* and *C*, for the 1931 and 1932 seasons, respectively. The Washington Navel oranges, shipped from San Dimas, Calif., on March 6, 1931, by unrefrigerated express, arrived at the cold-storage laboratory on March 11 and were immediately weighed into samples and placed at the various temperatures. Respiration determinations started on March 12 after the fruit had come to the desired temperatures. The runs at 32° F. were for 2-day periods, and those at the higher temperatures for 1-day periods. The Valencia oranges, also from San Dimas, were shipped by unrefrigerated express on June 29, 1932, arrived at the cold-storage laboratory on July 5, and were placed at 32° F. until used. Respiration determinations at temperatures of 32° to 60° were started on July 8; those at 70°, 90°, and 100°, on July 18; and those at 80°, on July 25. The runs at 32° and 40° were for periods of 2 days but are plotted as 1-day periods on the chart. At all other temperatures the runs were for 1-day periods.

As in Florida oranges, there was a decrease with time in the respiratory rates of the California Washington Navel and Valencia oranges at 70° to 100° F. (fig. 3, *A* and *C*). In the Washington Navel oranges there was also a slight decrease at 60° but some increase at 50° and less at 40°. In the Valencia oranges the rate remained fairly constant at 60° and 50°, with a slight increase at 40°. In both varieties the rate remained rather constant at 32°.

For the more recent experiments, the Washington Navel oranges were picked and shipped from Corona, Calif., on March 6, 1942, and arrived at the cold-storage laboratory on March 12. The oranges were kept at 32° F. until the determinations were started on March 17.

The fruit came from two different groves, and determinations were made on duplicate lots from each grove. The juice of the oranges from one grove averaged 13.0 percent of soluble solids and 1.22 percent of citric acid; that from the other grove averaged 11.9 and 1.03 percent of soluble solids and citric acid, respectively. Although the fruit from the two groves differed in the composition of the juice, it did not differ significantly in respiratory activity; the points on the curves (fig. 3, *B*) represent the averages for all four lots at each temperature.

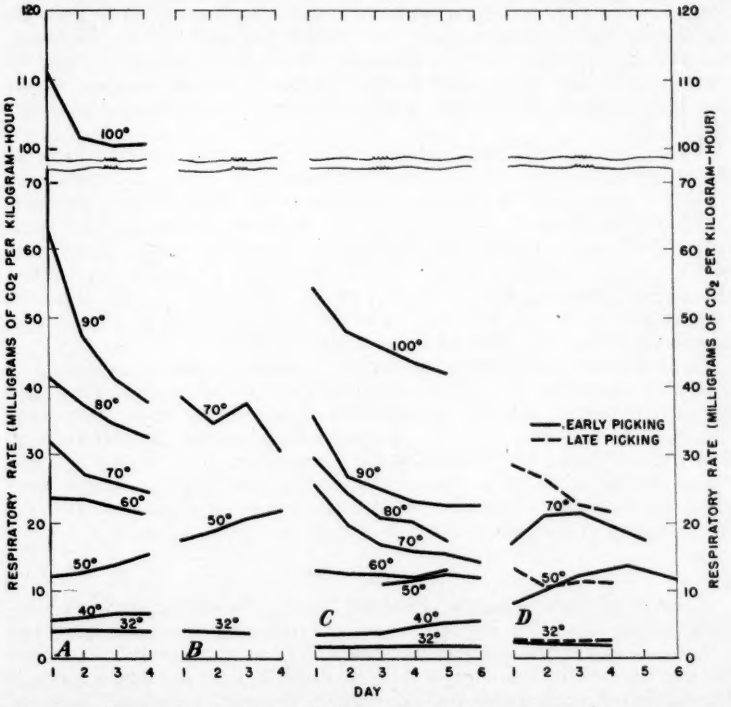


FIGURE 3.—Effect of temperature (° F.) on respiratory activity of oranges grown in California: *A*, Washington Navel oranges, 1931 season; *B*, Washington Navel oranges, 1942 season; *C*, Valencia oranges, 1932 season; *D*, early and late pickings of Valencia oranges, 1942 season.

As in the earlier tests, the rate of respiration remained constant at 32° F., increased with time at 50° and decreased at 70°. The average rate at 32° was 3.9 mg. of carbon dioxide per kilogram-hour, as in the 1931 season. However, at 50° and 70° the rate was considerably higher in 1942 than in 1931 (fig. 3, *A* and *B*).

Two pickings of Valencia oranges were made in a grove near Corona, Calif. The first picking, representing early midseason fruit, was made on July 7, 1942, was shipped by unrefrigerated express, and was received on July 14. The second picking, representing late maturity, was made from the same grove on October 1, 1942, and was received on October 7. The juice in oranges of the

first picking had 10.1 percent of soluble solids, 0.66 percent of citric acid, and a solids-acid ratio of 15.3. The late picking had 11.5 percent of soluble solids, 0.68 percent of acids, and a solids-acid ratio of 16.9. The late picking was somewhat higher in soluble solids than the first picking but did not differ appreciably from the first picking in respiratory activity except at 70° F., where it was considerably higher. At 32° and 50° the rates were approximately the same as the earlier (1932) results (fig. 3, *C* and *D*).

The average rate of respiration of the Washington Navel oranges from Florida (fig. 1, *B*) was about the same as that found in 1942 for California Navel oranges (fig. 3, *B*) at 32°, 50°, and 70° F. However, the averages for the Valencia oranges from California (fig. 3, *C* and *D*) were consistently lower than for the Florida Valencia oranges, except the late picking of Florida Valencia oranges, which was also low (fig. 1, *D*).

There was no significant difference in respiratory activity between Washington Navel oranges from Florida and early or midseason Valencia oranges from Florida. However, the Valencia oranges from California respired at a lower rate than the Washington Navel oranges from California. Gunn and Nedvidek [3] found the respiratory rates of California Valencia oranges at 70° and 100° F. to be even lower than those reported here. At 31° to 42° Trout et al. (*19*) recorded respiratory rates of Valencia and Washington Navel oranges in Australia about the same as those reported here.

In all the lots of oranges the carbon dioxide evolution increased to a greater extent at the higher temperatures (90° F. and above) than the oxygen intake, with a consequent increase in the respiratory ratio (fig. 2, *A*, *C*, and *D*). In all lots considerable intramolecular respiration was indicated by the high respiratory ratios at 100° and to a less extent by those at 90°. The ratios at the lower temperatures were generally between 1.33 and 1.00, but there was no consistent relation of the ratios to temperature below 90° in the various lots.

Florida Grapefruit

As with Florida oranges, different lots of Florida grapefruit were used during the 1929–30 season at the different temperatures, but samples of nearly all lots at 60° F. were used as standards. The data for the various lots are presented in table 2. There was a greater difference between duplicate samples of grapefruit than of oranges, doubtless because of the small number of fruit used per sample. Average respiratory rates for each temperature are shown in figure 4, *A*. As with oranges, the rate decreased with time at the higher temperatures, and the steepness of the curves generally increased with increased temperature. At 50° and below, there was relatively little change in rate with time.

The respiratory rate of these lots of grapefruit (table 2) at 32° F. averaged about 2.6 mg. of carbon dioxide per kilogram-hour and increased to nearly 70 mg. at 110°. As with oranges, the rate of carbon dioxide evolution increased with temperature to a greater extent than the oxygen intake, with a consequent increase in the ratio of carbon dioxide to oxygen (fig. 2, *E*). The respiratory ratio indicated some

[3] Gunn, A., and Nedvidek, R. D. Color investigation of citrus fruits. Studies on respiration of Valencia and Navel oranges. Calif. Fruit Growers Exch. Res. Dept. File 25, July to Dec. 1929. [1930.] [Processed.]

intramolecular respiration at 80°, increasing to a maximum at 100°, with a respiratory ratio of nearly 2.0.

TABLE 2.—*Effect of temperature and other factors on respiratory activity of Florida grapefruit, 1929–30 and 1942*

[Respiratory activity expressed as milligrams of CO_2 per kilogram-hour]

Lot No.[1]	Date	Respiratory activity (CO_2 evolved)[2] at indicated temperature (°F.)								
		32	40	50	60	70	80	90	100	110
	1929	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
	Dec. 3–7	-----	3.3	-----	10.0	-----	-----	-----	-----	-----
		-----		-----	11.6	-----	-----	-----	-----	-----
1	Dec. 9–14	2.0	-----	-----	10.0	-----	21.1	-----	-----	-----
		2.2	-----	-----	11.0	-----	16.9	-----	-----	-----
	Dec. 17–23	2.2	-----	-----	10.7	-----	-----	-----	-----	-----
		-----	-----	-----	10.1	-----	-----	-----	-----	54.6
	1930									
2	Jan. 6–13	2.2	-----	-----	11.4	-----	-----	-----	51.4	81.6
		2.1	-----	-----	12.7	-----	-----	-----	51.4	81.6
	Jan. 20–25	-----	-----	6.5	14.9	-----	-----	23.0	-----	-----
		-----	-----	7.3	10.5	-----	-----	27.5	-----	-----
3	Jan. 27–Feb. 2	-----	-----	-----	11.9	15.2	-----	31.1	-----	-----
		-----	-----	-----	14.0	14.0	-----	25.4	-----	-----
	Feb. 3–8	-----	-----	-----	16.8	18.0	-----	28.3	-----	-----
		-----	-----	-----	13.6	-----	-----	29.6	-----	-----
4	Feb. 10–17	-----	5.4	-----	15.3	-----	-----	-----	-----	-----
		-----	5.9	-----	16.1	-----	-----	-----	-----	-----
	Feb. 22–Mar. 3	-----	-----	-----	-----	19.5	-----	-----	-----	-----
		-----	-----	-----	-----	17.5	-----	-----	-----	-----
5	Mar. 3–8	-----	-----	-----	-----	14.7	-----	-----	-----	-----
		-----	-----	-----	-----	12.7	-----	-----	-----	-----
	Apr. 19–25	3.4	-----	-----	18.1	-----	-----	-----	-----	-----
		4.3	-----	-----	-----	-----	-----	-----	-----	-----
Average, 1929–30		2.6	4.5	6.9	13.2	16.0	19.0	27.5	51.4	68.1
	1942									
6a	Jan. 16–23	2.0	-----	8.4	-----	14.7	-----	-----	-----	-----
		2.0	-----	7.6	-----	12.0	-----	-----	-----	-----
6b	Jan. 27–Feb. 2	1.8	-----	7.5	-----	21.8	-----	-----	-----	-----
		1.9	-----	8.1	-----	16.9	-----	-----	-----	-----
7a	Jan. 16–23	1.8	-----	8.1	-----	14.3	-----	-----	-----	-----
		1.8	-----	8.8	-----	16.3	-----	-----	-----	-----
7b	Jan. 27–Feb. 2	1.7	-----	7.2	-----	18.0	-----	-----	-----	-----
		1.8	-----	6.1	-----	17.6	-----	-----	-----	-----
8	Feb. 17–23	1.9	-----	8.7	-----	24.1	-----	-----	-----	-----
		1.8	-----	9.0	-----	25.2	-----	-----	-----	-----
9	....do	2.4	-----	9.3	-----	26.0	-----	-----	-----	-----
		2.4	-----	9.7	-----	25.1	-----	-----	-----	-----
Average, Jan.–Feb. 1942		1.9	-----	8.2	-----	19.3	-----	-----	-----	-----

[1] Lot 1, grapefruit purchased on Washington, D. C., market, Dec. 3, 1929; variety and previous history unknown. Lot 2, grapefruit purchased on Washington, D. C., market, Jan. 2, 1930; shipped from Manavista, Fla.; variety unknown. Lot 3, grapefruit shipped directly from Florida; variety unknown. Lot 4, grapefruit bought on Washington, D. C., market, Jan. 10, 1930; fruit (Brogdexed) from Orlando, Fla. Lot 5, grapefruit shipped directly from Arcadia, Fla., Feb. 14, 1930. Lot 6, Marsh grapefruit grown in Portsmouth series (low hammock) soil, shipped directly from Vero Beach, Fla., Jan. 12, 1942. Lot 6a, on sour orange rootstock; solids, 10.0 percent; acid, 1.26 percent; ratio, 7.9. Lot 6b, on rough lemon rootstock grown in Norfolk fine sand; solids, 8.4 percent; acid, 1.26 percent; ratio, 6.7. Lot 7a, same as lot 6a but Duncan variety; solids, 10.2 percent; acid, 1.58 percent; ratio, 6.5. Lot 7b, same as lot 6b but Duncan variety; solids, 9.2 percent; acid, 1.33 percent; ratio, 6.9. Lot 8, Foster (pink) grapefruit on sour orange rootstock grown in Parkwood series soil, shipped from Bradenton, Fla., Feb. 11, 1942; solids, 10.3 percent; acid, 1.07 percent; ratio, 9.6. Lot 9, Thompson (pink) grapefruit, same as lot 8; solids, 9.0 percent; acid, 1.21 percent; ratio, 7.4.

[2] The 2 figures given for each date period represent determinations on duplicate samples of fruit.

Marsh (seedless) and Duncan (seeded) grapefruit were obtained from two sources in Florida. The first shipment of both varieties picked on January 12, 1942, was obtained from growers on the eastern coast of Florida near Vero Beach. Grapefruit from this section is

tender, thin-skinned, and of high quality. It was grown on sour orange rootstock in a low hammock soil which has a high organic-matter content. Another lot of the same varieties picked a week later was obtained from near Davenport, Fla. This was grown on rough lemon rootstock in a Norfolk fine sand which has a low organic-matter content. The results for these lots, together with those for two varieties of pink grapefruit (Foster and Thompson), are shown in table 2 and figure 4, *B* and *C*.

There was no consistent difference in respiratory activity between the two varieties Marsh and Duncan or because of the sources and

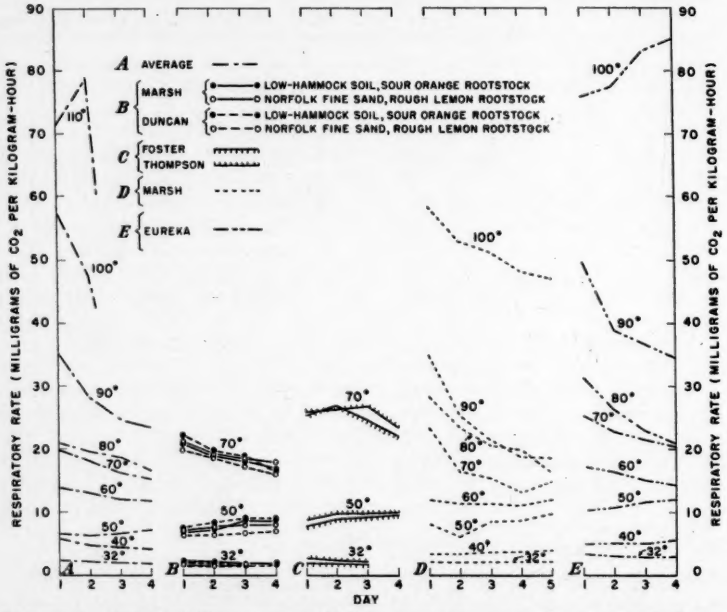


FIGURE 4.—Effect of temperature (°F.) on respiratory activity of grapefruit and lemons: *A*, Average for various lots of grapefruit grown in Florida, 1929–30 season; *B*, grapefruit grown in Florida, 1942 season; *C*, average for various lots of grapefruit from trees grown at Bradenton, Fla., on sour orange rootstocks, 1942 season; *D*, Marsh grapefruit grown in California, 1932 season; *E*, Eureka lemons grown in California.

rootstocks from which they were obtained, although in the fruit from Vero Beach the solids content was somewhat higher on the sour orange rootstock. The pink varieties (Foster and Thompson) respired at a slightly higher rate at 32° and 50° F. and at a considerably higher rate at 70° than did fruit of the Marsh and Duncan varieties. The higher rate for the pink varieties was not associated with a higher content of soluble solids or acidity in the juice of the fruit.

CALIFORNIA GRAPEFRUIT

The effect of temperature on the respiration of Marsh (seedless) grapefruit from California is shown in figure 4, *D*. This lot of grape-

fruit was shipped by unrefrigerated express from San Dimas on June 29, 1932, and arrived at the cold-storage laboratory on July 5. The fruit was stored at 32° F. until used. Samples were weighed and placed at temperatures of 32° to 60° on July 9, and respiration determinations were started on July 11. Other samples were weighed and placed at 70° and 90° on July 18, and determinations were started July 19. At 80° and 100° the corresponding dates were July 25 and 26. At the higher temperatures, the respiration rates (fig. 4, *D*) were similar to those for Florida grapefruit (fig. 4, *A*). With California grapefruit there was relatively little difference in respiratory activity between 80° and 90°, and with Florida grapefruit there was little difference between 70° and 80°. The respiratory ratio of the California grapefruit at 100° (fig. 2, *F*) was high (2.1), indicating considerable intramolecular respiration. The ratios at the lower temperatures did not correspond with those shown (fig. 2, *E*) for Florida grapefruit.

California Lemons

Five lots of Eureka lemons representing several stages of maturity were obtained from California in three different years (table 3). The light-green lemons of 1931 were shipped from San Dimas on April 10 and were received at the cold-storage laboratory 5 days later; the respiration determinations at 32° to 80° F. were begun the following day. At 90° and 100° the determinations were started on May 23 and 29, respectively, and the lemons were held at 32° until the experiments began. The silver lemons of 1931 were shipped from California on May 8 and received 5 days later. They were held at 32° until tested. Samples were weighed and placed at temperatures of 32° to 80° on May 15, and the determinations were started on May 18. At 90° and 100° the determinations were made at the same time as those with the light-green lemons. The tree-ripe lemons of 1932 were shipped from San Dimas about April 18, received April 23, and held at 40° until tested. At 32° to 70° the respiration determinations were started April 25, at 80° on April 27, and at 90° and 100° on May 5. Both the silver and the green lemons of 1933 were shipped from San Dimas on April 24. The fruit was received about 5 days later and held at 40° until tested. Respiration determinations on the silver lemons were started at all temperatures (32° to 90°) on May 15. With the green lemons, the determinations were started at 32° on May 15 and at the higher temperatures (40° to 90°) on May 22.

Although the respiration of lemons at several stages of maturity is shown in table 3, the only comparable lots are those of 1933, in which fruits of the two stages of maturity were obtained at the same time and from the same source. In these two lots the green lemons were found to respire in most cases more rapidly than the silver lemons at the various temperatures, on the basis of both carbon dioxide and oxygen determinations. The differences were statistically significant. The shipment of light-green lemons received in 1931 contained some that were dark green. The respiratory rates for these were determined at 60°, 80°, 90°, and 100° F. These data being incomplete with respect to temperatures used, are not presented in table 3. At the temperatures employed, the dark-green lemons respired consistently faster than the light-green lemons (average 9.1 percent higher). The results indicate that in lemons the

respiratory rate decreases with increased maturity. A similar relation has been shown for Valencia oranges picked at different times (table 1).

TABLE 3.—*Effect of temperature on respiratory activity of California Eureka lemons of various stages of maturity, 1931–33*

[Respiratory activity expressed as milligrams of CO_2 per kilogram-hour; O_2 measured as cubic centimeters per kilogram-hour]

Year	Maturity of fruit	Temperature	Respiratory activity (CO_2 evolved) during run[1] No. —					O_2 consumed, average (1–4)	Ratio of volume of CO_2 to volume of O_2 $\left(\frac{cc.\ CO_2}{cc.\ O_2}\right)$
			1	2	3	4	Average (1–4)		
		° F.	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Cubic centimeters*	
1931	Silver	32	2.8	2.7	2.6	2.6	2.7	1.1	1.25
1931	Light green	32	2.8	2.3	2.2	2.2	2.4	1.0	1.22
1932	Tree-ripe	32	2.5	2.2	2.0	2.3	2.2		
1933	Green	32	4.4	4.2	4.2	3.8	4.1	2.0	1.05
1933	Silver	32	4.5	4.0	3.6	3.7	3.9	1.9	1.05
Average		32	3.4	3.1	2.9	2.9	3.1	1.5	1.14
1931	Silver	40	3.8	3.8	3.2	3.9	3.7	1.6	1.18
1931	Light green	40	2.5	3.0	2.8	2.9	2.8	1.2	1.19
1932	Tree-ripe	40	3.0	3.0	3.1	3.7	3.2	1.4	1.17
1933	Green	40	9.0	8.5	8.2	8.6	8.6	3.2	1.37
1933	Silver	40	6.9	7.4	7.7	8.5	7.6	3.5	1.11
Average		40	5.0	5.1	5.0	5.5	5.2	2.2	1.20
1931	Silver	50	7.0	8.9	11.8	12.4	10.0	5.8	.88
1931	Light green	50	5.3	5.6	6.9	7.7	6.4	3.5	.93
1932	Tree-ripe	50	7.3	7.5	8.2	8.7	7.9	3.5	1.15
1933	Green	50	17.9	17.4	16.6	14.8	16.7	8.1	1.05
1933	Silver	50	14.6	14.5	14.3	15.8	14.8	6.9	1.09
Average		50	10.4	10.8	11.6	11.9	11.2	5.6	1.02
1931	Silver	60	12.7	13.5	13.9	14.0	13.5	7.0	.98
1931	Light green	60	8.9	10.4	11.2	11.6	10.5	6.0	.89
1932	Tree-ripe	60	14.5	14.3	13.3	13.9	14.0	6.7	1.07
1933	Green	60	28.6	24.5	20.0	16.8	22.5	11.3	1.02
1933	Silver	60	22.1	19.8	17.2	15.5	18.6	8.4	1.13
Average		60	17.4	16.5	15.1	14.4	15.8	7.9	1.02
1931	Silver	70	18.3	19.4	18.1	17.8	18.4	10.0	.94
1931	Light green	70	21.8	23.2	24.7	24.4	23.5	12.3	.97
1932	Tree-ripe	70	23.8	22.9	23.7	22.9	23.3	11.6	1.02
1933	Green	70	34.8	29.1	19.8	17.6	25.3	14.7	.88
1933	Silver	70	29.4	19.7	22.1	20.1	22.8	12.4	.94
Average		70	25.6	22.9	21.7	20.6	22.7	12.2	.95
1931	Silver	80	30.5	30.7	26.5	24.9	28.2	15.5	.93
1931	Light green	80	24.7	21.1	19.6	16.9	20.6	12.7	.83
1932	Tree-ripe	80	24.6	21.0	19.1	19.1	20.9	8.3	1.28
1933	Green	80	40.0	30.7	25.3	21.8	29.5	15.6	.96
1933	Silver	80	38.0	29.2	25.2	22.5	28.7	13.2	1.11
Average		80	31.6	26.5	23.1	21.0	25.6	13.1	1.02
1931	Silver	90	49.7	41.4	37.5	34.6	40.8	17.4	1.20
1931	Light green	90	62.4	52.7	50.8	50.1	54.0	23.9	1.15
1932	Tree-ripe	90	36.6	31.4	28.5	27.5	31.0	14.4	1.10
1933	Green	90	50.0	35.6	33.5	28.7	37.0	16.3	1.16
1933	Silver	90	52.3	35.0	33.5	32.6	38.3	15.2	1.28
Average		90	50.2	39.2	36.8	34.7	40.2	17.4	1.18
1931	Silver	100	71.9	78.5	85.6	85.1	80.3	30.0	1.36
1931	Light green	100	91.7	87.5	80.9	90.1	89.8	36.3	1.26
1932	Tree-ripe	100	63.7	66.3	74.1	81.1	71.3	24.2	1.50
Average		100	75.8	77.4	83.2	85.4	80.5	30.2	1.37

[1] Runs were for 1 day, except at 32° and 40° F., at which they were for 2 days.

In the 1933 season the lemons respired at a much higher average rate at 32° to 60° F. than in the two previous seasons (table 3). In some instances at the lower temperatures, the rate in 1933 was at least double the rates of the previous seasons. On the other hand, at 90° the rates in 1931 were for the most part higher than those in 1933. The reasons for these differences are not known, but probably they are due to some cultural or climatic factor.

Curves for the average rates at the different temperatures are shown in figure 4, *E*. Contrary to the results with the other citrus fruits, the rate of respiration of lemons at 100° F. increased with time. As with the other fruits, the rate decreased with time at 90° to 60°. At 50° there was a slight increase, and at 40° and 32° there was little or no change during the period studied. With increase in temperature there was a steady increase in respiratory rate, the increases in carbon dioxide and oxygen being closely parallel up to 80°. Above 80° the rates increased more rapidly, particularly in carbon dioxide (table 3).

The respiratory ratios approximated 1.0 between 50° and 80° F. (table 3 and fig. 2, *G*), with somewhat higher ratios above and below these points. As indicated previously, a ratio of 1.0 indicates the respiration of sugar. It is rather surprising to find a fruit with such a high acid content apparently respiring only sugar over such a wide range of temperatures. The respiratory ratio at 100° averaged only slightly above 1.33, indicating little or no intramolecular respiration at this temperature in lemons, although considerable intramolecular respiration was indicated in the other citrus fruits at this temperature. The smaller size of the lemon fruit, and consequently the greater surface area per unit of weight, may be responsible for the lack of intramolecular respiration, because the greater surface area would facilitate gaseous exchange.

TEMPERATURE COEFFICIENTS

Van't Hoff's temperature coefficient (Q_{10}) expresses the rate of increase in the velocity of a reaction with each increase in temperature of 10° C. (18° F.). Temperature coefficients of 2.0 to 3.0 or more are typical of chemical reactions, and Mathews (*12*) stated that between 10° and 40° C. (50° and 104° F.) nearly all vital activities are doubled or trebled by a rise in temperature of 10° C. A Q_{10} of less than 2.0 is more typical of a physical process. The Q_{10} of a chemical reaction is generally higher at low temperatures. The temperature coefficients for the respiration (carbon dioxide output and oxygen intake) of citrus fruits are shown for overlapping ranges (18° F.) of temperature in table 4 for the early results, in which a wider total range of temperatures was used, and in table 5 for later results of carbon dioxide evolved at 32° to 70°. At the lowest temperature range (32° to 50°) the rate generally increased more than three times (average 3.9 and 3.6 in the early results and 5.1 in the later results). The Q_{10} decreased with increased temperature and reached a minimum at 62° to 80° or 72° to 90°, with average minima of 1.5. At the higher temperatures (82° to 100° and 92° to 110°) the Q_{10} increased. The lower coefficients between 62° and 90°, particularly for oxygen intake, indicate that physical processes, possibly diffusion of the gases, control the rate of oxidation at this range of temperatures.

Trout et al. (*19*) indicated for oranges a Q_{10} of 4.0 from 36° to 59° and of 1.5 from 59° to 78°. These values are in good agreement with those shown in tables 4 and 5. Contrary to the results presented herein, Trout et al. reported a further decrease in Q_{10} to 1.0 between 78° and 90°.

TABLE 4.—*Temperature coefficients (Q_{10}) of respiration (carbon dioxide output and oxygen intake) of citrus fruits, 1931–32*

Temperature range (° F.)	Oranges						Grapefruit				Lemons		Average		
	Florida		California				Florida		California (Marsh)		California (Eureka)				
			Washington Navel		Valencia										
	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2 and O_2
	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}
32 to 50	3.9	2.8	3.3	4.2	6.0	5.2	2.7	2.0	4.0	3.7	3.7	3.7	3.9	3.6	3.8
42 to 60	2.8	2.7	2.9	3.4	2.1	2.2	2.6	2.3	2.4	2.1	2.5	2.8	2.5	2.6	2.6
52 to 70	2.2	2.2	1.8	1.8	1.5	1.8	2.2	1.9	1.9	2.3	1.9	2.1	1.9	2.0	2.0
62 to 80	1.6	1.8	1.5	1.2	1.6	1.6	1.4	1.2	1.7	1.7	1.5	1.2	1.5	1.5	1.5
72 to 90	1.5	1.1	1.6	1.3	1.4	1.3	1.6	1.3	1.3	1.2	1.7	1.4	1.5	1.3	1.4
82 to 100	1.8	1.2	2.7	1.6	2.0	1.4	2.3	1.8	2.3	1.3	1.9	2.2	2.2	1.6	1.9
92 to 110	2.0	2.0					2.2	2.7					2.1	2.3	2.2

HEAT OF RESPIRATION

In the respiration process, energy is released principally in the form of heat. The heat of respiration is generally computed from the carbon dioxide evolved, on the assumption that this is derived from the complete oxidation of a hexose sugar and that all the energy is released in the form of heat. It will be pointed out later that reactions other than the complete oxidation of sugar may and probably do occur and that the oxygen reading should be a more reliable measure of the energy or heat released.

The heat of respiration in British thermal units (B. t. u.) per ton of fruit per day is presented in tables 6 and 7. The heat of respiration was computed from the carbon dioxide evolved by multiplying the milligrams of carbon dioxide evolved per kilogram of fruit per hour by the factor 220. For the earlier results, in which a greater range of temperatures was used, the heat of respiration in British thermal units per ton-day was computed from the oxygen intake as well, by multiplying the cubic centimeters of oxygen intake per kilogram of fruit per hour by the factor 423.65. Thus the heat of respiration was directly proportional to the rate of respiration and varied as the respiratory rate varied. For oranges, as computed from CO_2 evolved, the heat of respiration ranged from 427 to 979 B. t. u. at 32° F., and increased to 22,660 B. t. u. at 100°. For lemons, the heat of respiration, similarly computed, ranged from an average of 675 B. t. u. at 32° to 17,710 at 100°. For grapefruit, the heat of respiration, similarly computed, ranged from 385 to 528 B. t. u. at 32° and increased to over 11,000 B. t. u. at 100°. At 32° to 80°, the average heat of respiration, when computed from the carbon dioxide determinations, was 14 to 27 percent higher than when computed from the oxygen determinations, and above 80° the difference was even greater (40 to 96 percent).

TABLE 5.—*Temperature coefficients (Q_{10}) of respiration (carbon dioxide output) of citrus fruits, 1941–42*

Temperature range (° F.)	Oranges												Grapefruit, Florida						Average
	Florida									California									
	Washington Navel	Parson Brown		Valencia						Washington Navel	Valencia		Marsh [1]		Duncan [1]		Foster	Thompson	
				Early picking		Midseason picking		Late picking			Early picking	Late picking	a	b	a	b			
		Sour orange	Rough lemon	Sour orange	Rough lemon	Sour orange	Rough lemon	Sour orange	Rough lemon										
	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}	Q_{10}
32 to 50	6.3	7.1	6.4	4.7	5.5	5.7	5.7	5.1	5.5	5.2	5.4	4.3	4.0	4.2	4.7	3.8	4.8	4.0	5.1
42 to 60	2.4	2.8	2.4	2.3	2.3	2.6	2.6	2.5	2.5	2.2	2.1	2.1	2.0	2.7	2.2	2.5	2.9	2.7	2.4
52 to 70	2.0	1.8	2.0	1.8	1.8	2.0	2.0	1.9	1.9	1.7	1.5	1.9	1.6	2.2	1.6	2.4	2.3	2.3	1.9

[1] a=Fruit from Vero Beach section on sour orange rootstock; b=fruit from Davenport section on rough lemon rootstock.

TABLE 6.—*Heat of respiration of citrus fruits at different temperatures, computed from respiratory activity determinations of 1929 to 1933*

[Expressed as B. t. u. per ton of fruit per day]

Temperature (°F.)	Oranges						Grapefruit				Lemons		Average of ratios $\left(\frac{a}{b}\right)$
	Florida		California				Florida		California, Marsh		California, Eureka		
	a [1]	b [2]	Washington Navel		Valencia		a [1]	b [2]	a [1]	b [2]	a [1]	b [2]	
			a [1]	b [2]	a [1]	b [2]							
	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u	
32	686	721	895	621	427	382	455	379	499	390	675	633	1.18
40	1,410	1,204	1,397	1,124	1,034	864	1,069	935	845	759	1,142	915	1.19
50	2,706	2,131	2,970	2,618	2,589	1,972	1,522	1,175	1,980	1,368	2,508	2,332	1.26
60	4,554	3,692	4,994	4,656	2,750	2,400	2,772	2,244	2,552	1,942	3,476	3,297	1.18
70	6,578	5,382	6,006	5,244	3,927	3,566	3,520	2,718	3,872	3,448	4,994	5,118	1.14
80	7,788	5,462	8,008	5,789	4,554	4,124	4,180	2,785	4,818	3,973	5,654	5,495	1.27
90	10,230	6,116	10,428	7,132	5,720	4,682	6,050	3,692	5,170	4,380	8,844	7,299	1.40
100	15,202	7,379	22,660	9,858	9,913	5,814	11,308	5,537	11,396	5,139	17,710	12,669	1.96
110	22,242	12,442	------	------	------	------	17,952	10,907	------	------	------	------	1.72

[1] Computed from CO_2 measurements (220 × mg. CO_2 per kilogram-hour), assuming complete oxidation of hexose sugar (glucose).

[2] Computed from O_2 measurements (423.65 × cc. O_2 per kilogram-hour), assuming complete oxidation of both hexose sugars (glucose and fructose) and citric acid.

TABLE 7.—*Heat of respiration of citrus fruits at 32°, 50°, and 70° F., based on 1941–42 determinations of carbon dioxide evolved*

[Expressed as B. t. u. per ton of fruit per day]

Temperature (°F.)	Oranges					Grapefruit			
	Florida			California		Florida			
	Washington Navel [1]	Parson Brown [2]	Valencia [3]	Washington Navel	Valencia [4]	Marsh [5]	Duncan [5]	Foster	Thompson
	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.	B. t. u.
32	616–660	440–473	451–979	858	495–627	407–440	385–396	407	528
50	3,938–4,070	2,805–3,366	2,464–4,598	4,334	2,552–2,706	1,716–1,760	1,463–1,859	1,947	2,090
70	8,360–9,020	5,707–6,732	5,313–9,119	7,755	4,048–5,896	2,937–4,257	3,366–3,916	5,423	5,621

[1] Range in B. t. u. values due to location of trees. (See table 1, lot 7.)

[2] Range in B. t. u. values due to rootstock (lower value for rough lemon and higher value for sour orange). (See table 1, lot 8.)

[3] Range in B. t. u. values due to rootstock and maturity (lower value for rough lemon picked late and higher value for sour orange picked early). (See table 1, lots 9, 10, and 11.)

[4] Range in B. t. u. values due to maturity. (See fig. 3, *D*.)

[5] Range in B. t. u. values due to rootstock and soil type. (See table 2.)

EFFECT OF ETHYLENE TREATMENTS ON RESPIRATION

METHODS

Ethylene, which is used for the degreening of citrus fruits, is usually applied at the rate of 1 part to 5,000 of air in intermittent charges or 1 part to 50,000 in continuous flow for 48 to 60 hours at 80° to 85° F. (*20*). In addition to destroying chlorophyll, ethylene greatly stimulates the respiratory activity of citrus (*1; 2; 3; 9; 17, pp. 42–44*) [4] and other fruits. It seems likely that the effect of ethylene on respiratory rate may be a measure also of the effect of ethylene on the rate of degreening.

In order to study the effect of ethylene on respiration, the gas was added to the respiration chambers after the fruit was sealed in the

[4] See also footnote 3, p. 334.

chambers. This was done by removing the funnel from the upper inlet tube and displacing the desired volume of ethylene into the tube and washing it into the chamber with water. In most instances the desired

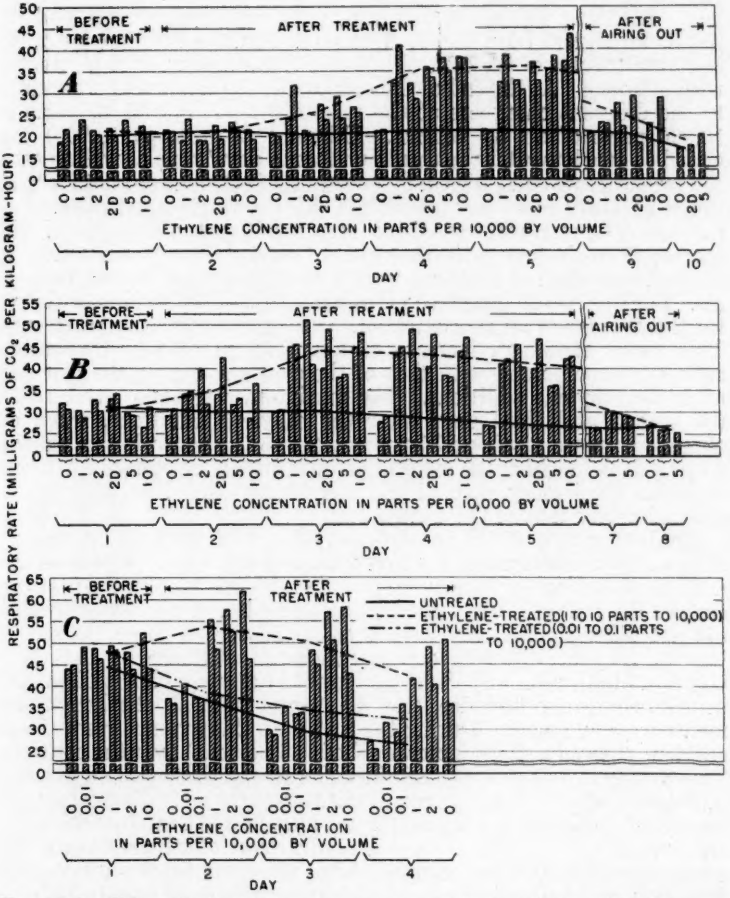


FIGURE 5.—Effect of ethylene treatments on respiratory activity of Florida-grown oranges at 60° F. (*A*), 70° (*B*), and 85° (*C*). The ethylene treatments were applied only once except for those marked 2D, in which successive applications were made daily for 4 days.

quantity of ethylene was introduced only once. In a few instances daily doses of ethylene were added for several days.

FRUITS TESTED

ORANGES

Figure 5 shows the results of a study of the effects of ethylene on the respiration of Florida oranges at 60°, 70°, and 85° F. The fruit

tested at 60° was shipped from Frostproof, Fla., on March 18, 1930, and was received 4 days later. It had not been treated previously with ethylene. Only four oranges were used in each respiration chamber, but the respiratory activity was determined on duplicate lots. Ethylene was not added during the first 2 days' runs, and the results for these 2 days (fig. 5, *A*) show no significant difference in the respiratory rates of the control lots and those that were to be treated with ethylene. At the end of the second day's run ethylene was added to the different chambers at the rate of 0, 1, 2, 5, and 10 parts to 10,000 parts of air by volume. Ethylene (2 parts to 10,000) was added to two lots after the third and fourth days' runs as well as after the second day's run. The daily additions of ethylene did not increase the respiratory rates any more than did the single application after the second run; nor was there any significant difference in the increased respiratory rates that could be attributed to differences in the concentration of ethylene from 1 to 10 parts per 10,000 parts of air. For this reason it seemed permissible to plot the respiratory curve for the average of all concentrations used in the ethylene treatments. This curve shows an increase of about 25 percent in respiratory activity, as compared with that of the controls, during the first day after the ethylene additions. The maximum rates were attained on the second and third days after the ethylene additions, when an average increase of about 70 percent above the controls was shown. After 2 days in which the respiration chambers were opened for airing out, the respiratory rate of the ethylene-treated lots dropped to approximately that of the control lots.

Similar results at 70° F. are shown in figure 5, *B*, for Valencia oranges shipped from Bradenton, Fla., on February 20, 1930. The determinations of respiratory rate were started on March 10. In the first run (before the ethylene was added) the respiration of lots to be treated did not differ significantly from that of the control lots. As at 60° (fig. 5, *A*), the different concentrations of ethylene or the daily addition of ethylene at 70° did not result in any significant difference in the respiratory response, and the results from all ethylene treatments were averaged. During the first day, this average response to ethylene showed a slight increase (about 15 percent) over the controls. The maximum response was attained the second day, with an average increase of about 47 percent in respiratory activity. Thereafter the respiratory rate of the ethylene-treated lots showed a gradual decrease, and even in the lots that were given daily doses of ethylene there was a corresponding decrease in rate. After the chambers were aired out for a day, the respiratory rate of the ethylene-treated lots dropped to approximately that of the controls and subsequently even below the controls.

The results of using ethylene at 85° F are shown in figure 5, *C*, for oranges shipped directly from Florida. The determinations were made May 12 to 16, 1930. In addition to concentrations of 1, 2, and 10 parts per 10,000 by volume, lower concentrations of 1 to 100,000 and 1 to 1,000,000 were used. During the first run, before the application of the differential treatments, the respiration of the control lots averaged somewhat lower than that of the lots to be treated. The untreated samples showed a rather rapid decrease in respiratory rate from nearly 45 mg. of carbon dioxide per kilogram-hour on the first day to about 26.5 mg. on the fourth day. The lots treated with the

lower ethylene concentrations (1 to 100,000 and 1 to 1,000,000) averaged somewhat higher in respiratory rate than the controls before treatment but showed a similar decrease in rate with time, indicating that such low concentrations of ethylene had no effect on the respiratory activity. Samples treated with the higher concentrations (1, 2, and 10 parts per 10,000) of ethylene showed a marked increase in respiratory rate during the first day after treatment, with a rather rapid decline thereafter but with the rate maintained considerably above that of the control lots.

Washington Navel oranges from San Dimas, Calif., were picked and shipped on March 6, and respiration determinations were started

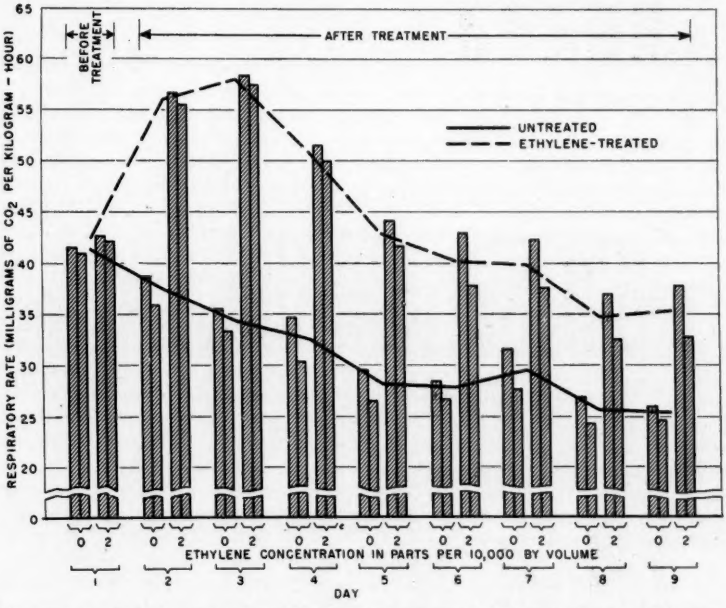


FIGURE 6.—Effect of ethylene treatments on respiratory activity at 80° F. of Washington Navel oranges grown in California.

March 12. Three oranges were tested at 80° F. in each respiration chamber and, as usual, determinations were made on duplicate lots. One concentration only (2 to 10,000) of ethylene was applied after the first run. The results are shown in figure 6.

During the first run the lots to be treated with ethylene averaged slightly higher in respiratory rate than the control lots. The control lots showed a rather rapid decrease in rate from 41.3 mg. of carbon dioxide per kilogram-hour on the first day to 25.3 mg. on the ninth day. The ethylene-treated lots showed a marked increase in respiratory activity during the first day after the introduction of the ethylene, and averaged 50 percent greater than the control lots. There was a further increase in the respiratory rates of the treated lots during the second day after treatment, when these lots averaged 69 percent

greater than the controls. A sharp decline in the respiratory rate of the treated lots followed, but on the seventh day after treatment these lots averaged 36 percent higher in respiratory rate than the untreated lots.

Grapefruit

As with oranges, the respiratory activity of grapefruit as influenced by treatments with ethylene was determined at 60°, 70°, and 85° F.

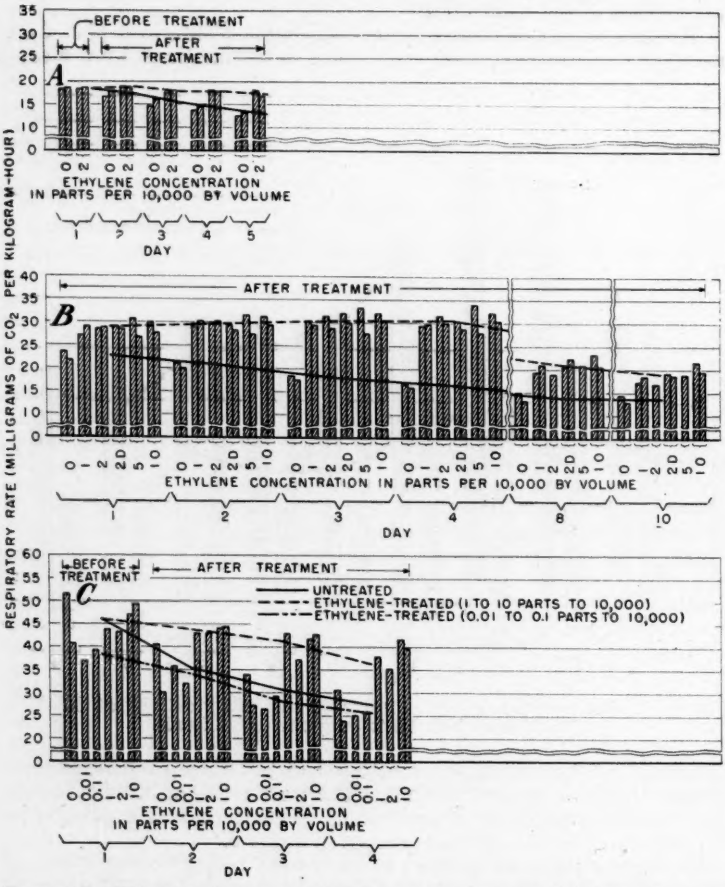


FIGURE 7.—Effect of ethylene treatments on respiratory activity of Florida-grown grapefruit at 60° F. (*A*), 70° (*B*), and 85° (*C*). The ethylene treatments were applied only once except for those marked 2D, in which successive applications were made daily for 4 days.

The fruit tested at 60° was shipped from Orlando, Fla., and had been waxed by the Brogdex process. It was purchased on the Washington, D. C., market on January 10, 1930. Results of the ethylene treatments on this fruit are shown in figure 7, *A*. The untreated fruit

(without ethylene) showed a gradual decline in respiratory activity from 18.4 to 12.8 mg. of carbon dioxide per kilogram-hour, whereas the respiratory activity of the treated fruit remained nearly constant and was about 36 percent higher than that of the untreated fruit by the fourth day after treatment. In commercial practice the ethylene treatment would be applied before the waxing, so these results may not be representative of commercial conditions.

The fruit tested at 70° F. was shipped directly from Arcadia, Fla., on February 14, and the determinations were started on February 24, 1930. As with oranges, there was no significant difference between the different concentrations of ethylene (within the limits of 1 to 10 parts per 10,000) in their effects on the respiratory rates (fig. 7, *B*). The daily addition of ethylene for 4 days did not increase the rate more than a single addition at the beginning of the experiment. Consequently, results with all of the ethylene-treated fruit were averaged for comparison with those from fruit receiving no ethylene. The untreated lots showed a gradual decline in respiratory activity from 22.5 mg. of carbon dioxide per kilogram-hour on the first day to 16.3 mg. on the fourth day. The respiratory activity of the ethylene-treated lots averaged 26.5 percent greater on the first day and reached a maximum increase on the fourth day of 87 percent, with a respiratory activity of 30.5 mg. of carbon dioxide per kilogram-hour. After several days in which the fruit was aired out, the rate of respiration of the treated lots decreased greatly but was still about 37 percent greater than that of the controls 10 days after the ethylene was added and 6 days after the fruit was aired out.

The fruit tested at 85° F. was shipped directly from Florida on February 20, 1930, and had not been treated with ethylene. It was held at 32° until the beginning of the experiment on May 5, 1930. The results (fig. 7, *C*) show a rapid decrease in the respiratory rate of the untreated lots from an average of 46 mg. of carbon dioxide per kilogram-hour on the first day to 27.2 mg. on the fourth day. The lots that were treated with the lower concentrations of ethylene (1 to 100,000 and 1 to 1,000,000) averaged lower in respiratory rate than the control lots on the first day (before they were treated). Although they did not decrease in rate as greatly as the controls on the first day after treatment, the difference in rate was not significant. The lots treated with the higher concentrations (1, 2, and 10 parts per 10,000) of ethylene averaged the same as the control lots before the treatments. After treatment their respiratory rates decreased but more slowly than those of the controls, and averaged 24 percent higher on the first day after treatment and 42 percent higher on the third day after treatment.

Lemons

The respiratory rate of Eureka lemons at 80° F., as influenced by ethylene, is shown in figure 8. The dark- and light-green lemons were shipped from San Dimas, Calif., by nonrefrigerated express on April 10 and received on April 15; the respiration determinations were started on April 16, 1931. The silver lemons were shipped by express from San Dimas on May 8, 1931, and received May 13; the determinations were started May 18. As the silver lemons were picked at a much later date, they were not directly comparable with the less mature lemons but showed a similar response to the ethylene treatment.

Therefore, results obtained with fruit of all three stages of maturity were averaged to determine the influence of ethylene on respiration.

In the first run (before the ethylene was added) the lots to be treated averaged somewhat higher in respiratory activity (15 percent) than the control lots. The lemons showed a greater response to ethylene treatment than either oranges or grapefruit. The respiratory

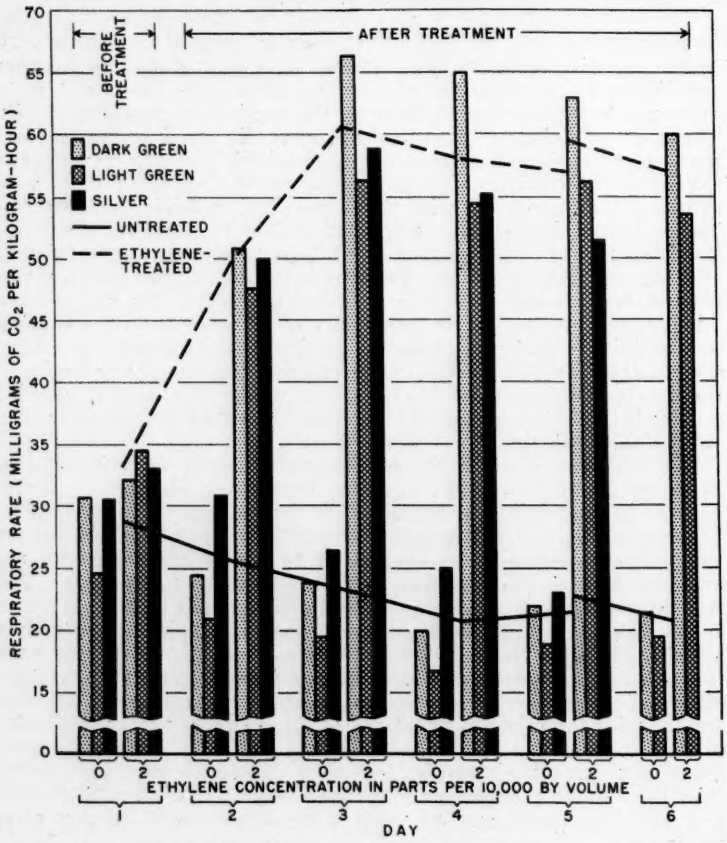


FIGURE 8.—Effect of maturity and ethylene treatments on respiratory activity at 80° F. of Eureka lemons grown in California.

rates of the treated lemons increased from 33.2 mg. of carbon dioxide per kilogram-hour before treatment to 49.8 mg. on the first day after treatment and reached a peak of 60.7 mg. on the second day after treatment. This represented an increase over the untreated lots of 96 percent the first day after treatment and 160 percent the second day. Five days after treatment with ethylene, the treated lots (green lots only) were respiring at a rate 178 percent higher than that of the control lots.

EFFECT OF DECAY ON RESPIRATION OF LEMONS

In a number of instances the respiratory activity of certain lots of fruit increased greatly and was out of line with the previous trend. An example of this is shown in figure 1, *C*, at 70° F. An examination of such lots generally disclosed the presence of decay in one or more of the fruits, and such results were usually discarded. In one instance slight mold on a single fruit apparently resulted in large increases in the carbon dioxide evolved. In order to determine the

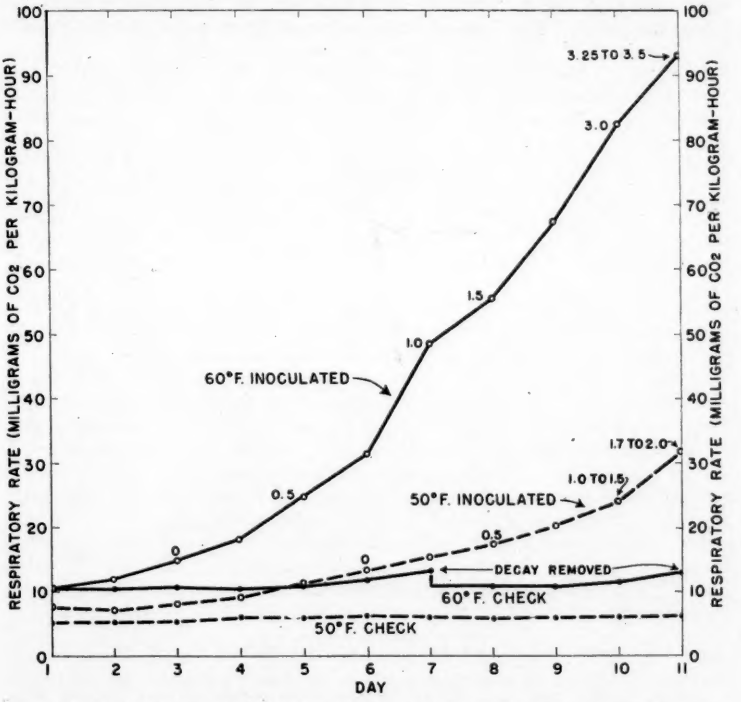


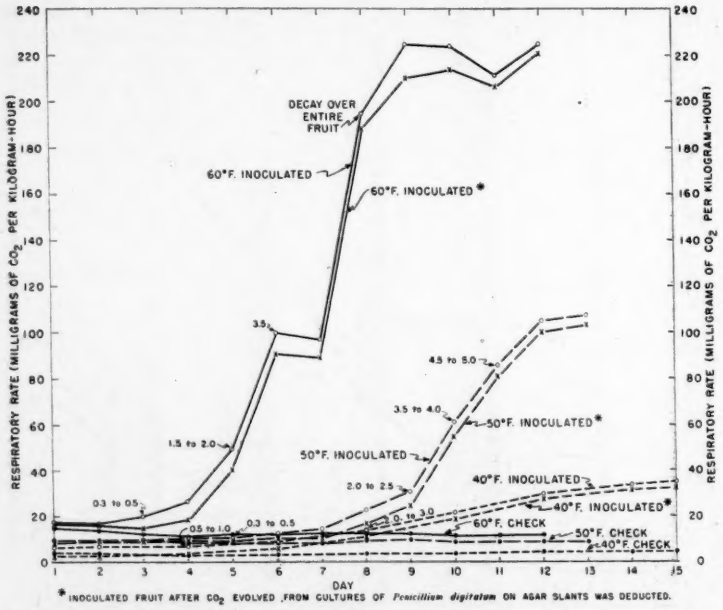
FIGURE 9.—Effect of inoculations with *Penicillium digitatum*, causing green mold decay, on respiratory activity at 60° and 50° F. of California-grown Eureka lemons, 1931. Numbers on curves represent estimated diameter (inches) of lesions.

extent to which decay might increase the carbon dioxide evolved, lemons were inoculated with cultures of green mold (*Penicillium digitatum* Sacc.) and the black rot fungus (*Alternaria citri* Ell. and Pierce). The lemons for the first experiment were shipped from California on July 2, and the experiment was started on July 15, 1931. The respiratory activity of the fruit was determined at 40°, 50°, and 60°.

The results of the experiment are shown in figure 9. During the course of the experiment no decay developed at 40°F, and therefore no data for 40° are presented in the figure. *Alternaria citri* did not

develop at any of the temperatures, and the results for this fungus also are omitted. At 50° (fig. 9) the respiratory activity of the inoculated fruit started off considerably higher than that of the untreated checks. After 6 days the inoculated lemons were respiring at double the rate of the controls, though no evidence of decay lesions was apparent. By the eleventh day decay lesions about 1.75 to 2 inches in diameter had developed, and the carbon dioxide evolved by the decaying lemons was over five times that of the controls.

At 60° F., after 11 days the decay lesions were 3.25 to 3.5 inches in diameter and the respiratory rate was 93 mg. of carbon dioxide per kilogram-hour. The sound lemons generally respired at the rate of



* INOCULATED FRUIT AFTER CO_2 EVOLVED FROM CULTURES OF *Penicillium digitatum* ON AGAR SLANTS WAS DEDUCTED.

FIGURE 10.—Effect of inoculations with *Penicillium digitatum* on respiratory activity at 60°, 50°, and 40° F. of California-grown Eureka lemons, 1932. Numbers on curves represent estimated diameter (inches) of lesions.

about 11 mg. of carbon dioxide per kilogram-hour. However, slight decay developed in the check fruit on two occasions and increased the respiratory activity somewhat. The highest rate attained by the inoculated fruit at 60° was more than eight times the average rate of the control fruit. No doubt a considerably higher rate would have been attained by the inoculated fruit if the experiment had continued, because the trend was still steeply upward when the test was terminated.

A second experiment to determine the effect of decay on respiration was made on full-ripe lemons from San Dimas, Calif., received April 23, 1932, and held at 40° F. until the beginning of the experiment on May 4, 1932. As decay had not developed at 40° in the first experi-

ment, the inoculated lemons in the second experiment were incubated for 3 days at 60° before being transferred to 40°. To determine whether wounding would exert a stimulating effect on respiration, one each of the duplicate uninoculated lots of fruit at 50° and 60° was punctured with a sterile needle; thus the uninoculated fruit was wounded in the same manner as the inoculated fruit. The wounding had no apparent effect on respiration, and the curves in figures 9 and 10 are based on averages of the duplicate determinations.

The first experiment had not shown what part of the stimulation in respiratory activity of the decaying fruit was due to the fruit and what part to the decay organism; so in the second experiment an equal number of potato-dextrose agar slants in test tubes were inoculated at the same time that the lemons were inoculated and the carbon dioxide evolved by the cultures was determined separately (fig. 10). This was deducted from the total carbon dioxide evolved by the decaying lemons to get the net effect of the decay on the respiratory activity of the lemons. It is recognized that the lemon fruit may supply a more or a less favorable medium for the organism so that it may grow and respire more or less rapidly on the fruit than on the culture medium; hence the carbon dioxide evolved on the culture medium may only approximate that given off by the organism growing on the fruit.

The results, presented in figure 10, show a stimulation of carbon dioxide evolution similar to that shown in figure 9. On the ninth day after inoculation the respiratory activity of the decaying fruit at 60° F. reached a maximum of over 220 mg. of carbon dioxide per kilogram-hour, as compared with only about 12 mg. for the sound fruit. After the carbon dioxide evolved by the agar cultures was deducted, the respiratory rate of the inoculated fruit reached a maximum of over 210 mg. of carbon dioxide, or more than 17 times that of the controls. At 50° the respiratory activity of the inoculated lemons did not begin to increase sharply until 7 to 8 days after inoculation, whereas at 60° there was an increase after 3 to 4 days. The inoculated fruit at 50° reached a respiratory rate of over 100 mg. of carbon dioxide per kilogram-hour by the twelfth and thirteenth days and this was about the maximum. At both 50° and 60° the maximum respiratory activity of the decaying fruit was attained at about the time that decay had completely covered the surface of the fruit. The maximum respiratory activity attained at 50° by the inoculated fruit was about 12 times that of the sound fruit. At 40° the decaying lemons showed a more gradual increase in carbon dioxide evolved than at the higher temperatures. After 16 days the rate of carbon dioxide evolution of the inoculated fruit was still increasing, so that the maximum probably had not been attained. At this time, about 75 percent of the surface showed decay and the respiratory rate was over 8 times that of the control fruit.

The results shown in figure 10 indicate that the carbon dioxide evolved by the decay organism was responsible for a relatively small percentage of the increase in carbon dioxide evolved by decaying lemons. Recently Biale (*1*) has shown that the vapor from lemons decaying because of *Penicillium digitatum* stimulated the respiratory activity of sound lemons exposed to the vapor from the decaying lemons. The stimulation amounted to 50 to 100 percent and was

attributed to the evolution of ethylene by the decay organism. Miller et al. (*15*) showed that various citrus fruits, including lemons, produce epinasty in test plants, indicating the evolution of ethylene. They showed further that decaying fruit inoculated with *P. digitatum* produced epinasty more rapidly than sound fruit and that agar cultures of *P. digitatum* also produced epinasty. Biale (*1*) reported that vapor from other decay organisms (*P. italicum*, *Sclerotinia sclerotium*, and *Aspergillus niger*) had no stimulatory effects on respiration. Since the maximum stimulatory effect of ethylene on lemons was less than 200 percent (fig. 8), this would account for only a fraction of the increased respiratory activity observed in decaying lemons (figs. 9 and 10). It seems likely that the disorganization of the tissue resulting from decay would tend to greatly increase the activity of the hydrolytic and catabolic processes, and this may account in large measure for the great increase in carbon dioxide evolved. The observed increase in respiration of lemons is much greater than the increase of 100 percent observed by Kidd (*10*) in apples.

RESPIRATION OF FLESH OF PEELED LEMONS

The rind of citrus fruits, particularly that of lemons, represents a rather large proportion of the total weight of the fruit. Kudryavzeva (*11*) observed that the sugar and acid remained fairly constant in the flesh of mandarin oranges during storage but that there was considerable dry matter lost from the rind. He suggested that in respiration only the materials in the rind were used. Trout et al. (*19*) also observed that the respiration of oranges was more closely related to the composition of the rind than to that of the juice. Data presented by Miller and Schomer (*14*) show the percentage of total sugar in the rind of lemons to be double that in the flesh, with more lost from the rind than from the flesh during storage. These analyses indicate that in determinations of respiratory activity of citrus fruits the results are influenced more by physiological reactions in the rind than in the flesh.

It was found that with care lemons could be peeled so as to leave a very thin layer of albedo, without injury to the flesh. When exposed to the fairly dry atmosphere of the laboratory the thin layer of albedo dried and formed a thin parchmentlike skin on the lemon, with no apparent wilting of the flesh. Lemons peeled in this manner were used to determine the respiratory activity of the flesh alone. The results in table 8 show that the rind constituted about a third of the total weight of the fruit. In order to maintain a low relative humidity, and thus retard decay on the peeled fruit, concentrated sulfuric acid was placed in the bottom of the respiration chambers. In spite of this precaution, 60 percent of the peeled fruit showed decay (mostly slight) when removed after 5 days in the chambers. For this reason the determinations for only 3 days were used, as there was a large increase in respiratory activity in the peeled fruit on the fourth and fifth days, apparently because of the decay. The data in table 8 indicate that the flesh respires in proportion to its weight at practically the same rate as the whole fruit. However, the evidence is not conclusive, as there may have been some stimulatory effect of the rather severe wounding on the respiratory rate of the peeled lemons.

TABLE 8.—*Respiratory activity of whole and peeled lemons*

[Expressed as milligrams of CO_2 per kilogram-hour]

Treatment	Total lemons	Weight		CO_2 evolved			
		With peel	Without peel	First day	Second day	Third day	Average
	Number	*Grams*	*Grams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Not peeled	15	1,630.5		17.2	13.6	12.3	14.4
Peeled	15	1,586.5	1,071.0	16.1	14.2	13.8	14.7

EFFECT OF ARSENATE SPRAY ON RESPIRATION OF GRAPEFRUIT

Citrus fruit from trees sprayed with arsenicals has been found to be lower in acid content than fruit from trees not so sprayed. Since arsenical sprays apparently have a rather marked physiological effect on the fruit it was desirable to study its effect on respiration. For this purpose Marsh and Duncan grapefruits that had been sprayed with lead arsenate (1 pound to 100 gallons) were shipped from Florida on April 8, 1943, and the respiratory activity was determined at 70° F. There was no appreciable difference in the amount of soluble solids in the juice between fruit from sprayed trees and that from unsprayed trees. Spraying reduced the acidity from 1.21 to 1.14 gm. per 100 ml. of juice in the Marsh variety and from 1.55 to 1.38 gm. per 100 ml. in the Duncan variety. The effect of spraying on the respiratory activity of the fruit at 70° is shown in table 9. Spraying reduced the average respiratory activity of both varieties. The reduction was only about 8 percent for Marsh but was nearly 20 percent for Duncan. The effect of spraying with arsenicals appears to be to lower the metabolic activity of the fruit as measured by respiratory rate. This is associated with lower acidity of the juice.

TABLE 9.—*Effect of spraying trees with lead arsenate (1 pound to 100 gallons) on the respiratory activity at 70° F. of grapefruit grown in Florida*

[Expressed as milligrams of CO_2 per kilogram-hour]

Variety	Treatment	CO_2 evolved in lot—				Average
		1	2	3	4	
		Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
Marsh	Not sprayed	19.6		20.0	23.6	21.1
	Sprayed	17.6	15.9	23.9	20.8	19.5
Duncan	Not sprayed	19.3	23.5	19.4	19.5	20.4
	Sprayed	15.8	15.2	16.8	18.0	16.5

DISCUSSION

The results of the study of the effect of ethylene on the respiration of oranges, grapefruit, and lemons are in general agreement with those reported by others in showing a marked increase in respiratory activity by a concentration of 1 to 10 parts of ethylene to 10,000 of air, followed by a decrease to or below that of untreated fruit. Denny (*2*, *3*) found that concentrations of 1 to 100,000 and 1 to 1,000,000 increased the respiratory activity of lemons. These relatively weak concentrations were used with oranges (fig. 5, *C*) and grapefruit (fig. 7, *C*), but with no significant effect on respiration.

If respiratory activity is an indication of the degreening activity of ethylene, then concentrations of 1 part or less of ethylene to 100,000 parts of air would not be effective for degreening oranges or grapefruit.

On the other hand, the respiratory activity would indicate that 1 part of ethylene in 10,000 of air was as effective as 10 parts per 10,000 and that an initial exposure to ethylene in a confined space was as effective as daily additions of ethylene.

The complete oxidation of a hexose sugar to carbon dioxide and water results in a respiratory ratio of 1.0; a respiratory ratio of 1.33 is obtained from the complete oxidation of citric acid, according to the following equations in which the heat produced is based on gram-molecular weights:

(1) $\underset{\text{Hexose sugar}}{2C_6H_{12}O_6} + 12O_2 \rightarrow 12CO_2 + 12H_2O + 1{,}348{,}600 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{12}{12} = 1.0$$

(2) $\underset{\text{Citric acid}}{2C_6H_8O_7} + 9O_2 \rightarrow 12CO_2 + 12H_2O + 949{,}000 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{12}{9} = 1.33$$

Respiratory ratios between 1.0 and 1.33 might represent the respiration of different proportions of sugar and citric acid, according to table 10, assuming that the carbon dioxide evolved and the oxygen consumed are due to the complete oxidation of these materials. However, it has been shown (*15*) that citrus fruit may evolve ethylene, presumably in the process of respiration. This might be evolved from the oxidation of a hexose sugar, as in the following equation, and would result in a respiratory ratio of 1.33.

(3) $\underset{\text{Hexose sugar}}{2C_6H_{12}O_6} + 6O_2 \rightarrow 8CO_2 + 8H_2O + \underset{\text{Ethylene}}{2C_2H_4} + 685{,}400 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{6} = 1.33$$

TABLE 10.—*Respiratory ratios obtained by complete oxidation of various proportions of hexose sugars and citric acid*

Hexose sugars	Citric acid	Respiratory ratio	Hexose sugars	Citric acid	Respiratory ratio
Moles	*Moles*		*Moles*	*Moles*	
100	0	1.00	40	60	1.18
90	10	1.025	30	70	1.21
80	20	1.05	20	80	1.25
70	30	1.08	10	90	1.29
60	40	1.11	0	100	1.33
50	50	1.14			

Alcohol has also been found in ripening fruit, particularly in apples (*4*, *13*, *18*), and might be formed in the partial oxidation of a hexose sugar or by intramolecular respiration, as in the following equations:

(4) $\underset{\text{Hexose sugar}}{2C_6H_{12}O_6} + 6O_2 \rightarrow 8CO_2 + 6H_2O + \underset{\text{Alcohol}}{2C_2H_5OH} + 693{,}400 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{6} = 1.33$$

(5) $\underset{\text{Hexose sugar}}{2C_6H_{12}O_6} \rightarrow 4CO_2 + \underset{\text{Alcohol}}{4C_2H_5OH} + 38{,}200 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{4}{0} = \infty$$

Thus a respiratory ratio of 1.33 may represent the partial oxidation of a hexose sugar, with the formation of either alcohol or ethylene as one of the end products, as well as the complete oxidation of citric acid.

With respiratory ratios above 1.33, it is probable that a reaction such as that shown in equation 5 enters into the respiration. Thus, a respiratory ratio of 2.0, such as was obtained at 100° F. with Florida oranges (fig. 2, *A*), might represent the intramolecular respiration of 3 moles of a hexose sugar (as in equation 5) and the complete oxidation of 1 mole of a hexose sugar or 2 moles of citric acid (as in equations 1 and 2). Such an equation follows:

(6) $\underset{\text{Hexose sugar}}{3C_6H_{12}O_6} + 12O_2 \rightarrow 12CO_2 + 12H_2O + \underset{\text{Alcohol}}{12C_2H_5OH} + 1{,}463{,}200 \text{ cal.}$

$$\text{Respiratory rate} = \frac{CO_2}{O_2} = \frac{12}{12} = 2.00$$

Acetaldehyde has also been reported (*13*, *14*, *18*) as an end product in the ripening of apple and lemon fruits and might be formed as in the following equation:

(7) $\underset{\text{Hexose sugar}}{2C_6H_{12}O_6} + 7O_2 \rightarrow 8CO_2 + 8H_2O + \underset{\text{Acetaldehyde}}{2C_2H_4O} + 790{,}600 \text{ cal.}$

$$\text{Respiratory ratio} = \frac{CO_2}{O_2} = \frac{8}{7} = 1.14$$

This would result in a respiratory ratio equivalent to the complete oxidation of equal molecular weights of a hexose sugar and citric acid (table 10).

From the materials that are known to be present in the fruit and have been shown to occur during the ripening processes, the foregoing reactions might reasonably be expected to represent those occurring during respiration; other reactions doubtless occur also. From these equations it is apparent that increases in the respiratory ratios above 1.0 may be due to other types of respiration than a change from sugar to acid as the material being respired. None of the reactions just indicated results in respiratory ratios above 1.33, except those (equations 5 and 6) in which intramolecular respiration takes place. It seems likely, therefore, that ratios above 1.33 indicate that intramolecular respiration accounts for part of the carbon dioxide evolved.

In intramolecular respiration (equation 5) only one-third as much carbon dioxide is formed as by the complete oxidation of the sugar (equation 1). Thus, a unit of carbon dioxide from intramolecular respiration represents the break-down of three times as much sugar as an equal amount of carbon dioxide produced by the complete oxidation of the sugar, or carbon dioxide evolved at a respiratory ratio of 2.0 (equation 6) might represent the break-down of twice as much sugar as at a ratio of 1.0.

The numbers of calories given in equations 1 and 2, for the complete oxidation of hexose sugars and citric acid, were taken from Hodgman (*8*). The heat of combustion for fructose is slightly greater (0.38 percent) than that for dextrose, and the average for the two is given. The numbers of calories for the other equations, in which intermediate products were formed, were obtained by deducting the heat of combustion of the intermediate product from

the heat of combustion of complete oxidation. In equation 3, for example, the heat of combustion of 2 gram molecules of ethylene is 663,200 calories. This deducted from the heat of combustion of 2 gram molecules of sugar (1,348,600−663,200) gives 685,400 calories as the heat of combustion for equation 3, in which ethylene is formed.

The heat of respiration has generally been computed from carbon dioxide measurements, on the assumption that the carbon dioxide was evolved from the complete oxidation of the hexose sugar dextrose (*16*), as in equation 1. Haller et al. (6) have pointed out that this introduces an error of about 30 percent if the carbon dioxide comes from the complete oxidation of citric or malic acid. Since considerable acid is generally lost during ripening after harvest, it is likely that much of the carbon dioxide comes from the oxidation of the acid, with a proportionate error in the computation of the heat of respiration. Similarly, if the carbon dioxide comes from the partial oxidation of the sugar, as in equations 3, 4, and 7, the actual heat of respiration would be much lower (12 to 24 percent) than the calculated heat of respiration (table 11). With partial intramolecular respiration, as in equation 6, the error would be very large (46 percent).

TABLE 11.—*Heat of respiration per unit of CO_2 evolved and O_2 consumed from various probable respiratory reactions*

Equation No.	Heat of respiration per unit of—		Equation No.	Heat of respiration per unit of—	
	CO_2	O_2		CO_2	O_2
	Calories	*Calories*		*Calories*	*Calories*
1	112,383	112,383	5	9,550	
2	79,083	105,444	6	60,967	121,933
3	85,675	114,233	7	98,825	112,943
4	86,675	115,567			

On the other hand, the heat evolved per unit of oxygen consumed varied relatively little (table 11), regardless of the type of reaction involved. If the complete oxidation of a hexose sugar (equation 1) is again used as a standard for calculating the heat of respiration, the errors due to the type of reaction would be relatively slight when computed from the oxygen measurements. The lowest heat of respiration or combustion per unit of oxygen results from the complete oxidation of citric acid (table 11, equation 2), and this was only 6.4 percent lower than the standard when calculated from the oxygen determinations, as contrasted with a 30-percent reduction when calculated from the carbon dioxide determinations. The highest heat of respiration per unit of oxygen was obtained with intramolecular respiration, and with a respiratory ratio of 2.0 (as in equation 6) the actual heat of respiration would be only 8.5 percent higher than the standard if computed from oxygen determinations, as contrasted with results 46.0 percent lower than the standard if computed from carbon dioxide determinations.

From these considerations it is apparent that the heat of respiration as generally computed from carbon dioxide determinations may be highly erroneous unless the reaction involved in its production is known and the computation is based on the specific reaction in-

volved. On the other hand, the heat of respiration per unit of oxygen consumed is fairly constant, regardless of the type of reaction concerned. This emphasizes the importance of determining the oxygen consumed, as well as the carbon dioxide evolved, in respiration determinations in which the calculation of the heat of respiration is of interest.

However, in connection with these calculations, attention should be given to the results obtained by Green et al. (*5, pp. 20–21*) during an investigation of a number of fruits and vegetables held at 45° and 65° F., in which they calculated the heat of respiration from the carbon dioxide evolved and determined the heat by calorimetric measurements. They concluded that ". . . the values for the heat produced which are obtained by calculations based on the rate of production of carbon dioxide are within 10 percent of those obtained by means of the calorimeter herein described." With two lots of sound oranges they found the calculated values to be only 3 and 5 percent higher than those obtained by means of the calorimeter. Their results do not show divergencies between calculated and measured heat of respiration as great as those indicated from theoretical consideration of possible reactions (table 11). It is likely that respiration in citrus fruits usually consists of not one but several of the reactions indicated, with the complete oxidation of a hexose sugar (equation 1) predominating, so that the heat of respiration calculated from the carbon dioxide determinations would not differ greatly from the actual heat evolved. The error due to such calculations is not likely to be great, except at high temperatures at which intramolecular respiration occurs.

SUMMARY

The carbon dioxide evolved and the oxygen consumed by oranges, grapefruit, and lemons were determined under different experimental conditions.

The respiratory rates of all the fruits increased greatly with increased temperature. The temperature coefficients (Q_{10}) were highest between 32° and 50° F.; dropped to minima, averaging about 1.5 between 62° and 90°; and increased again above these temperatures, particularly in carbon dioxide output.

At the lower temperatures (32°, 40°, and 50° F.), the respiratory rates remained fairly constant with time, whereas at the higher temperatures (70° to 110°) the rates generally decreased with time. The respiratory ratios $\frac{\text{volume of } CO_2}{\text{volume of } O_2}$ did not differ significantly at the low and intermediate temperatures (32° to 80°) but increased markedly at the higher temperatures (90° to 110°), indicating intramolecular respiration.

The results indicate that the respiratory activity of oranges and lemons decreases with increased maturity.

The respiratory activity of oranges from trees on sour orange rootstocks was higher than that of oranges from trees on rough lemon rootstocks.

The heat of respiration of the different fruits was computed for the different temperatures. The heat of respiration averaged 11 to 96 percent higher when computed from carbon dioxide determinations

than when computed from oxygen determinations. It is pointed out that oxygen determinations represent the sounder basis for computing the heat of respiration, particularly at high temperatures.

The addition of ethylene, at the rates of 1 to 10 parts by volume to 10,000 parts of air, greatly increased the respiratory activity of oranges, grapefruit, and lemons. The increase was as much with 1 as with 10 parts of ethylene per 10,000; much weaker concentrations (1 part to 100,000 and 1 part to 1,000,000) had no apparent effect. Daily additions of ethylene at the rate of 2 parts per 10,000 did not increase or maintain the respiratory activity more than the initial addition of 2 parts per 10,000. The maximum respiratory activity was generally attained 2 to 3 days after the addition of ethylene; the activity then decreased; and respiration returned to normal after aeration.

The respiratory activity of lemons infected with green mold (*Penicillium digitatum*) was increased to as much as 12 times that of sound lemons.

The results indicated that respiratory activity occurs in both the flesh and rind of lemons approximately in proportion to their fresh weights.

The respiratory activity of grapefruit from trees sprayed with lead arsenate was lower than that of grapefruit from trees not so sprayed.

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RETENTION OF CAROTENE IN ALFALFA STORED IN ATMOSPHERES OF LOW OXYGEN CONTENT[1]

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INTRODUCTION

Partly because of its relatively high carotene content, alfalfa has long been recognized as of exceptional value in animal nutrition. Alfalfa meal as a source of carotene, or provitamin A, has increased in importance with increased demands for this vitamin and the threatened shortage of such concentrates as fishliver oils. Obviously preservation of the carotene content of alfalfa is important both in securing the greatest benefits from its use as a feedstuff and in economical recovery of the carotene.

Artificial drying or dehydration has been found effective in minimizing the losses of carotene that ordinarily accompany the curing of alfalfa in the field, but both the field-cured and the dehydrated product lose carotene rapidly under present-day methods of storage in air.

It is generally recognized that the loss of carotene in alfalfa is an oxidation process,[2] and numerous investigations have been made of the relations of time and temperature to the extent of carotene loss in air. But very little consideration has been given to the obviously important factor of oxygen exclusion in alfalfa storage. Taylor and Russell[3] found that storage of alfalfa meal in vacuo at approximately 0° C. was very effective in reducing carotene loss. Samples of alfalfa ground in a Wiley mill to pass a 1-mm. screen and containing 0.014 percent of carotene, when stored for 12 to 20 months in vacuo at $0 \pm 5°$ C. had a high degree of carotene stability. There was a loss of 23 percent in 12 months. Samples more finely ground in a ball mill, under the same conditions, suffered very little change in caretone throughout a 20-month period. On the other hand, a sample (Wiley-milled) kept in the dark at $0 \pm 5°$, in a sealed bottle containing air, lost 70 percent. Moreover, samples kept in the dark at room temperature, in air in sealed bottles, lost 87 percent (Wiley-milled) and 77 percent (ball-milled). It was thus shown that vacuum storage was very effective in preserving the carotene content of alfalfa.

Vacuum storage did not appear to be economically feasible, but storage in a gas that is free from, or low in, oxygen seemed likely to produce the same result as vacuum storage, at less expense. Controlled gas atmospheres are used commercially in the storage of fruit.

A search of the literature on gas storage and on refrigerated gas storage revealed that much has been published with respect to such

[1] Received for publication March 27, 1944.

[2] WILLSTÄTTER, R., and ESCHER, H. H. ÜBER DEN FARBSTOFFE DER TOMATE. Hoppe-Seyler's Ztschr. f. Physiol. Chem. 64: [47]—61. 1910. (See pp. 56-58.)

[3] TAYLOR, M. W., and RUSSELL, W. C. THE STABILITY OF CAROTENE IN PLANT TISSUES. Jour. Nutr. 16: 1-13, illus. 1938.

storage of fruits, vegetables, and meats; but no method was found in use or suggested for the commercial storage of alfalfa in an inert atmosphere or one of limited oxygen content. Accordingly the present investigation was undertaken to establish a basis for pilot-plant studies.

During the progress of this investigation the work of Wilder and Bethke[4] was published. These investigators found that the carotene in machine-dried alfalfa meal was stable when stored in sealed cans in vacuo or in an atmosphere of nitrogen at room temperature and at 37° C. Little or no loss of carotene occurred.

The investigation of Taylor and Russell had shown that cold storage reduced carotene loss in air in sealed containers from 87 percent at room temperature to 70 percent at approximately 0° C. It had further shown the effectiveness of vacuum storage in conserving carotene. The work of Wilder and Bethke confirmed the results of the former investigators with respect to storage in vacuo, and further indicated the effectiveness of storage in nitrogen at room temperature and at 37° C. In the report of their investigations, however, Wilder and Bethke do not state the extent of oxygen exclusion by evacuation or by replacement of air with nitrogen.

PLAN OF THE INVESTIGATION

Since the purpose of the present investigation was to provide a basis for commercial application, it was essential to acquire quantitative information on the degree of oxygen exclusion required for preservation of carotene. For this reason the investigation as originally planned had for its object a comparison of carotene losses in samples of alfalfa meal stored in closed containers containing atmospheres of different oxygen content, ranging from air to nitrogen containing 0.3 percent of oxygen, under as nearly identical conditions of temperature and relative humidity as possible. A temperature of 40° C. and a relative humidity of 40 percent were selected as typical of those generally prevailing in summer in the valleys of California and some other alfalfa-producing areas. Later the investigation was expanded to include the effect of carbon dioxide in the storage atmosphere, because this gas might be used as the so-called inert gas or as a constituent of other commercial inert gases. Also determination of the effect of a daily renewal of the storage atmosphere was included.

The three phases of experimentation carried out were as follows:

(1) Losses of carotene in alfalfa meal in closed containers in air [5] and in atmospheres consisting of nitrogen and 5.3 percent oxygen, 3.0 percent oxygen, 1.6 percent oxygen, and 0.3 percent oxygen, at 40° C. and a relative humidity of approximately 40 percent.

(2) The effect of 13 percent of carbon dioxide in the 3-percent-oxygen atmosphere. (The remainder was nitrogen.)

(3) The effect of a daily renewal of the 3-percent-oxygen atmosphere containing the 13 percent of carbon dioxide. This experiment may be considered as simulating conditions in commercial storage in an inert gas; i. e., considerable leakage of the gas and entrance of outside air.

[4] WILDER, O. H. M., and BETHKE, R. M. THE LOSS OF CAROTENE IN MACHINE-DRIED ALFALFA MEAL UNDER VARIABLE CONDITIONS OF STORAGE. Poultry Sci. 20: 304–312, illus. 1941.

[5] A preliminary comparison of carotene losses in air at 40° and 25° C. under these conditions had been made. At 40° the samples of a low-grade alfalfa meal lost 45 percent of the carotene in 67 days; at 25° samples of the same meal lost 30 percent in 69 days.

EXPERIMENTAL PROCEDURE

ALFALFA MEAL, PRELIMINARY TREATMENT, AND SAMPLING

The alfalfa meal [6] used in the experiments was prepared from dehydrated alfalfa on August 1, 1941. The alfalfa (fourth cutting) was about 30 days old when cut. Practically all the meal, on screening test, passed through an 18-mesh screen, 65 percent through a 60-mesh, 34 percent through a 120-mesh, and 19 percent through a 200-mesh. As received, on August 4, the meal contained 8.3 percent of moisture and had a carotene content of approximately 225 parts per million.

In order to preserve the carotene content and to promote the establishment of moisture equilibrium at the relative humidity selected for the experimental storage atmospheres, a sufficient quantity of the meal for all anticipated requirements was stored at 0° C., in uncovered dishes placed in large desiccators over sulfuric acid of 46.9-percent strength. From this common source, the meal was removed as required for the tests.

The containers used for the samples in storage were glass U tubes, Schwartz type. These were tested against leakage under moderate pressure. The volumetric capacity of the eight tubes used for storage in nitrogen varied from 26.2 to 28.1 cc. For storage in the other atmospheres the volumes ranged from a minimum of 19.8 to a maximum of 25.1 cc. The weights of samples placed in the tubes were all in the same proportion of meal to volumetric capacity, i. e., almost exactly 1 gm. of meal per 4.78 cc. of volume. A rough estimate showed that the loose meal occupied nearly 60 percent of the enclosed space.

A uniform procedure was followed in introducing the required quantities of samples into the U-tubes. The tubes were weighed, filled, and reweighed before the stopcocks were greased. The calculated quantity of meal required for a given tube was first weighed out in a covered weighing bottle to within 0.1 gm. This was then transferred carefully through a paper funnel into the previously weighed tube. Reweighing of the tube gave the exact weight of sample transferred. The stopcocks were then greased lightly with vaseline and the tube was reweighed. This last weight was used in determining any subsequent changes in weight before and at the conclusion of storage.

For each series of experiments, in air, nitrogen, etc., 12 samples of meal in the same number of U tubes were prepared in the manner described. All of these samples were subjected to the same treatment up to the beginning of storage. Four of the samples were then separated, 2 being used for determinations of moisture and 2 for carotene determinations at the beginning of storage. The remaining 8 samples were used for the various periods of storage.

THE APPARATUS

The apparatus used in the preparation of the gas mixtures and for the conditioning of the alfalfa samples for storage is shown in figures 1 and 2. In figure 1, *A* and *B* are 5-gallon bottles filled with water and connected as shown. *D* is a constant temperature water bath with heater, stirrer, bimetallic regulator, contrivance for adjusting inflow and outflow of running water (at a temperature below 23° C.),

[6] Obtained through the courtesy of Dr. George Kernohan, of Poultry Producers of Central California.

and the humidifier *E*. This humidifier is a specially constructed gas-washing bottle of spiral type filled with water. *O* is an electric oven in which the U tubes are placed, connected in series and attached to the apparatus at *h* and *k*. *W* is a wash bottle containing approximately 2 inches of water for saturating the emerging gas before it passes through the wet test meter (*M*). The pressure in the system is indicated by a small manometer (*p*).

When the gas desired for the storage atmosphere was air or nitrogen, this was introduced directly into the bottle *A* through stopcock *d* by way of *e* by displacement of the water into *C*, a large aspirator bottle. The other gas mixtures required contained a lower proportion of oxygen than that in air. They were prepared by introducing into *A* from an additional measuring bottle equal volumes of air and

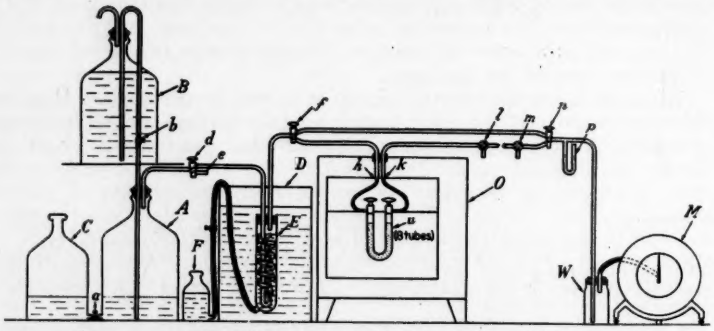

FIGURE 1.—Apparatus for gas aspiration and humidity control.

nitrogen, thus producing a gas containing approximately 10 percent of oxygen. Then successive dilutions of this mixture with nitrogen were made by the same procedure. For the preparation of the atmosphere of carbon dioxide, oxygen, and nitrogen it was found simpler to introduce nitrogen into bottle *A* to a previously determined level of water corresponding to a known volume of gas and to follow this by the introduction of the volumes of oxygen and carbon dioxide required for the concentrations desired. In operation, the gas in bottle *A* was displaced by water from bottle *B* through stopcock *b*.

PREPARING THE SAMPLES FOR STORAGE IN THE DIFFERENT ATMOSPHERES

A relative humidity of approximately 40 percent at 40° C. was desired for the storage atmospheres. The preliminary storage of the meal at 0° C. in desiccators over sulfuric acid of the proper strength tended to contribute to this end. After it was sampled and weighed into the U tubes, the meal, surrounded by air, was subjected to two operations. First, the 12 tubes were connected in series and 7 liters of air, conditioned by passage through gas-washing bottles containing sulfuric acid of 48.4 percent strength, was passed through the tubes for a period of approximately 6 hours. Previous experiments had indicated that by this operation at room temperature (approximately 25° C.) practically constant weights of the samples were attained. However, an average relative humidity of 46.5 percent instead of 40 percent for the atmospheres at the end of two such operations was found experimentally. This higher relative humidity

can be explained by the observed fact that in the determinations of relative humidity there was a small net loss in the moisture content of the alfalfa samples.

The apparatus used in this operation is shown in figure 2. *A* is a long glass tube containing sulfuric acid of approximately 48 percent strength set up for control of air pressure in *B* and *C*, two gas-washing bottles containing sulfuric acid of 48.4 percent strength. *T* is a U tube filled with glass wool to trap any spray of acid from *C*, and *U* represents the series of 12 U tubes. At the completion of this operation all the tubes were again weighed. Two of the samples were used for the determination of moisture and 2 for the carotene analysis. The moisture and carotene content of these samples was assumed to be applicable to each of the remaining 8 samples.

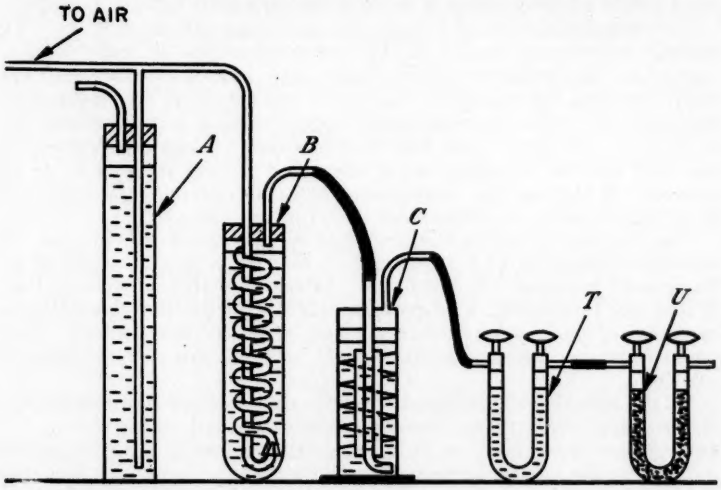

FIGURE 2.—Apparatus for preconditioning alfalfa to 40 percent relative humidity at room temperature.

The second operation consisted in placing the eight samples in the oven at 40° C. and establishing the desired atmosphere in the tubes. Previous tests had shown that air could be displaced in the eight tubes by the passage of 2.6 liters of nitrogen in 2 hours. As an added precaution, 4 liters of the desired atmosphere was passed in 3 to 4 hours.

The procedure followed for the production of a relative humidity of approximately 40 percent at 40° C. was based on the calculation that a gas saturated with water vapor at 23.8° C. and then heated to 40° should have a relative humidity of 40 percent at the higher temperature. This calculation did not take into account any change in the moisture equilibrium of the alfalfa at the higher temperature. A determination of the relative humidity produced by this procedure was made and it was found to be 45 percent. Here, again, as at room temperature, a small loss in total moisture of the samples was noted at the completion of the determination.

It would have proved detrimental to the accuracy of the determinations of carotene loss in storage to have made determinations of

relative humidity of the atmospheres in which the samples were actually stored, and consequently it can only be assumed that under identical conditions of procedure the same relative humidity prevailed in the atmospheres of all samples.

Before passage of gas through the samples was begun in *O* (fig. 1), the humidifier (*E*) was filled with water to displace air by raising the leveling bottle (*F*). The displaced air was by-passed through W and *M* by the use of the three-way stopcocks, *f* and *n*. The gas was then drawn in from *A* by lowering *F* and displaced in the same manner several times to flush out air in the apparatus between stopcocks *d* and *f*. The water in *E* was then lowered approximately 1½ inches from the rubber stopper, thus drawing in fresh gas, and the screw cock between *E* and *F* was closed. The desired rate of gas flow was established by by-passing it from *A* through stopcocks *d*, *f*, and *n*.

The temperature of the bath (*D*) was adjusted to 23.8° C. The sample tubes were placed in the oven, connected in series, and attached to the apparatus at *h* and *k*. The rate of gas flow was kept fairly uniform by control of the water level in *B* and adjustment of stopcock *d*. After the passage of approximately 4 liters of gas in 3 to 4 hours, the flow of gas was discontinued. For a few seconds the gas was allowed to escape at *l* through a drying tube to bring the pressure of the storage atmosphere to atmospheric. The stopcocks of all the tubes in the oven were then quickly closed.

The last-described procedure was slightly modified for the gas mixture containing 13 percent carbon dioxide, 3 percent oxygen, and 84 percent nitrogen. In this case, instead of water in bottles *A* and *B* and the humidifier, a 20-percent solution of sodium chloride saturated with the gas mixture was used, and the temperature of the water bath was maintained at 26.6° C. to take care of vapor-pressure relations.

In the experiment designed to show the effect of daily renewal of the storage atmosphere, the same concentrations of carbon dioxide and oxygen were used as in the preceding experiment with carbon dioxide in the closed containers. The procedure was also the same except that daily except Sundays a volume of the gas equal to twice the capacity of the tubes in the oven was passed through in the manner described.

At selected intervals, from 14 to 112 days, comparable for the respective atmospheres, samples were removed from the oven, weighed for comparison with original weights, and analyzed for carotene.

THE DETERMINATION OF CAROTENE

Carotene was determined by a slight modification of the method described by Kernohan.[7] Because of its simplicity and the comparatively brief time required, the method was thoroughly investigated and found to meet fully the requirements for consistent and precise determinations of small changes in the carotene content of the alfalfa.

The method specifies overnight digestion of the sample with petroleum ether. A longer digestion (48 hours) was found to yield slightly higher values for carotene (1 to 7 parts per million higher). In the first experiments the filtering column of soda ash used was 3½ inches

[7] KERNOHAN, G. THE DETERMINATION OF CAROTENE. Science 90 : 623. 1939.

long, but the results were unsatisfactory because not all of the non-carotene coloring matter was removed. The use of a 6-inch column eliminated this inaccuracy, and the increase in amount of absorbent caused no retention of carotene by the soda ash when a Skellysolve solution of pure carotene was filtered.

The procedure adopted is as follows: Digest the alfalfa sample (1.0–1.3 gm.) at room temperature for 48 hours with 100 cc. of Skellysolve. Filter the solution through a 6-inch column of soda ash. Wash the filter first with 50 to 60 cc. of the solvent and then with

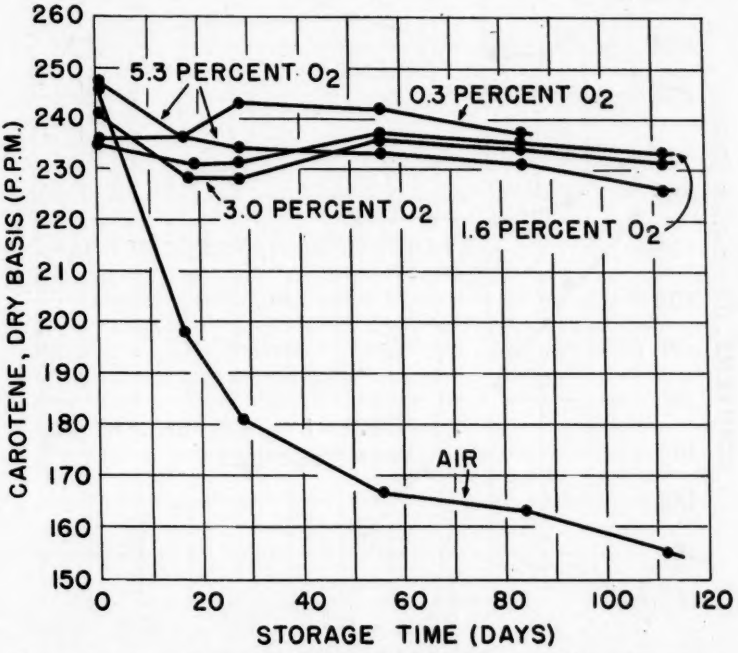

FIGURE 3.—Losses in carotene content of alfalfa meal stored in nitrogen containing various amounts of oxygen. (For the computed probable errors at individual points on the curves see table 1.)

35- to 40-cc. portions until the 250-cc. volumetric flask is filled to the mark, using slight air pressure upon the top of the column. Determine carotene by the use of a photoelectric colorimeter calibrated against solutions of pure carotene. In the writers' determinations, an Evelyn photoelectric colorimeter with a 420-mμ filter was used. The 6-inch filtering column had a diameter of 47 mm. The weight of soda ash (grade 58 percent, light) required for a column of these dimensions varied somewhat with the fineness of the soda ash (120 to 150 gm.).

RESULTS

The results of the storage tests are shown in tables 1 and 2 and graphically in figures 3 and 4. These tests were begun in successive

weeks. They were conducted first in air, and then in mixtures of nitrogen and oxygen with progressively decreasing proportions of oxygen (table 1); 5 months after the beginning, the tests with carbon dioxide were started (table 2). This fact accounts for the variation in original carotene values in the different series. The moisture content of the alfalfa samples ranged from 8.1 to 8.6 percent.

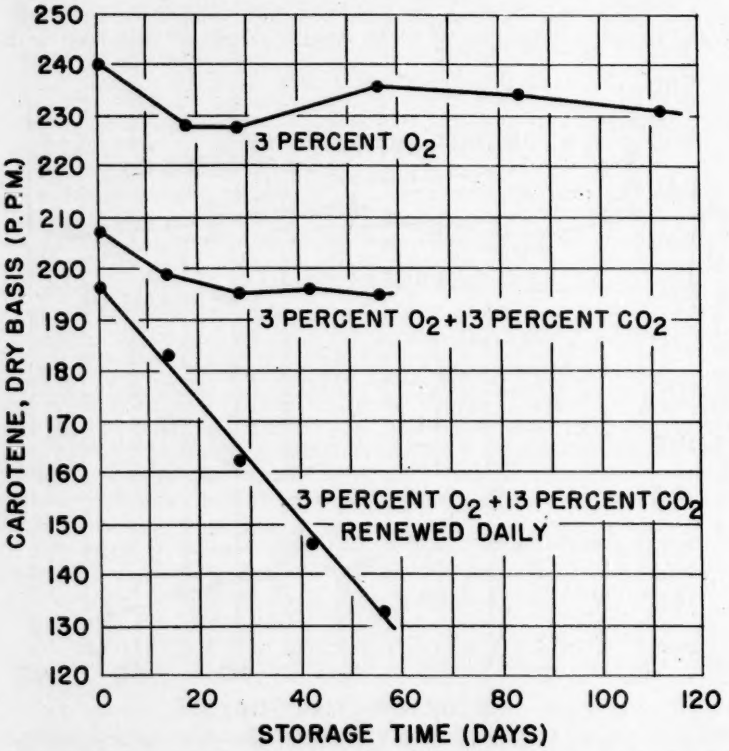

FIGURE 4.—Losses in carotene content of alfalfa meal stored in nitrogen containing various amounts of carbon dioxide and oxygen, with and without daily renewal of atmosphere. (For the computed probable errors at individual points on the curves see table 2.)

The data given in table 1 and figure 3 show definitely that a reduction in the oxygen content of the storage atmosphere tends to preserve the carotene in alfalfa. In air there was a progressive loss of carotene from 19.8 percent in 17 days to 37.3 percent in 112 days. In an atmosphere containing 5.3 percent of oxygen, the loss of 4.8 percent in 17 days increased to 8.9 percent in 112 days. With 3 percent oxygen, the loss of carotene reached its maximum of 5.8 percent in 18 days. The same loss was recorded at 28 days. The loss at 56 days had decreased to 2.5 percent, after which the loss was progressive up to 4.6 percent in 112 days.

TABLE 1.—*Carotene content of alfalfa meal and losses during storage in atmospheres of different oxygen content*

Length of storage (days)	Carotene content for storage atmosphere of—														
	Air			Oxygen, 5.3 percent; nitrogen, 94.7 percent			Oxygen, 3 percent; nitrogen, 97 percent			Oxygen, 1.6 percent; nitrogen, 98.4 percent			Oxygen, 0.3 percent; nitrogen, 99.7 percent		
	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss	Dry basis [1]	Probable error [2]	Loss
	P. p. m.	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*
0	[3] 252	6	0	[3] 248	6	0	[3] 238	5	0	234	3	0	236	3	0
	[3] 242			[3] 248			[3] 246			236			235		
17	198	7	19.8	[4] 236	1	4.8	[4] 228	4	[5] 5.8	[4] 231	3	[6] 1.7	[4] 236	4	0
28	181	3	26.7	236	5	5.7	230	3	5.8	234	3	1.7	244	4	+3.0
	181			231			227			229			241		
56	166	3	32.8	234	5	6.1	[3] 234	4	2.5	238	3	+.9	241	3	+2.5
	166			231			238			236			243		
84	167	4	34.0	232	4	6.9	232	6	3.3	236	3	0	236	8	+.4
	159			229			237			234			238		
112	155	4	37.3	[4] 226	4	8.9	[4] 231	5	4.6	[4] 233	9	.9			

[1] Average of 3 determinations on contents of same sample tube, except where indicated.

[2] Probable error in determination of carotene computed from Peters' formula: $R=\frac{0.845}{\sqrt{n(n-1)}}\Sigma v$, where n=number of observations and v=individual deviation from arithmetical mean.

[3] Average of 2 determinations on contents of same sample tube.

[4] Average of 4 determinations on contents of same sample tube.

[5] 18 days' storage.

[6] 19 days' storage.

TABLE 2.—*Carotene content of alfalfa meal and losses during storage in atmospheres consisting of various amounts of nitrogen, carbon dioxide, and oxygen, with and without daily renewal of atmosphere*

Length of storage (days)	Carotene content for storage atmospheres of—											
	(From table 1) Air			(From table 1) Oxygen, 3 percent; nitrogen, 97 percent			Oxygen, 3 percent; carbon dioxide, 13 percent; nitrogen, 84 percent			Oxygen, 3 percent; carbon dioxide, 13 percent; nitrogen, 84 percent—renewed daily		
	Dry basis	Probable error [1]	Loss	Dry basis	Probable error [1]	Loss	Dry basis [2]	Probable error [1]	Loss	Dry basis [2]	Probable error [1]	Loss
	P. p. m.	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*	*P. p. m.*	*P. p. m.*	*Pct.*
0	247	6	0	242	5	0	211	4	0	197	2	0
							204			197		
14							201	3	4.3	185	2	7.1
							197			181		
28	181	3	26.7	228	3	5.8	194	1	6.3	162	2	17.8
							195			161		
							198	3	5.8	147	2	25.9
							193			145		
56	166	3	32.8	236	4	2.5	194	3	6.3	133	3	32.5
							195			132		

[1] Probable error in determination of carotene computed from Peters' formula: $R=\frac{0.845}{\sqrt{n(n-1)}}\Sigma x$, where n=number of observations and x=individual deviation from arithmetical mean.

[2] Average of 3 determinations on contents of same sample tube.

In an atmosphere of 1.6 percent oxygen a maximum loss of 1.7 percent occurred in 19 and 28 days. After 28 days,the results were parallel to those of the 3.0 percent-oxygen atmosphere, where there was an apparent increase in carotene, followed by a decrease to a final loss of one-half that of 19 days. In nitrogen containing 0.3 percent oxygen no loss whatever was found. On the other hand, there was an apparent increase in carotene content between 17 and 28 days of 3.0 percent, which increase was reduced to 0.4 percent at the end of 84 days.

The deviations from progressive carotene loss are shown in figure 3. All the curves follow the same general pattern. For storage in nitrogen (containing 0.3 percent oxygen), the curve begins its upward trend at 17 days; from 28 days the trend is definitely downward. For 1.6

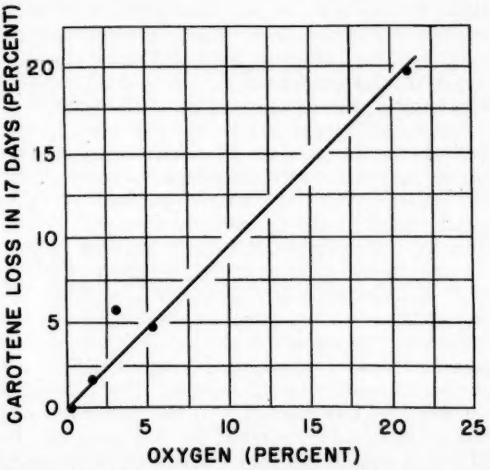

Figure 5.—Relation of initial carotene loss to oxygen content of storage atmosphere.

percent oxygen the trend upward begins at 28 days and downward at 56 days. The same is true for 3.0 percent oxygen. For 5.3 percent oxygen, although there is no definite upward trend at any time, from 28 days to 112 days a reduced rate of carotene loss is indicated. Even for air, after 56 days, the curve follows somewhat the same pattern as for 5.3 percent oxygen.

Table 2 and figure 4 show the effect of the presence of carbon dioxide in an atmosphere of 3.0 percent oxygen, and also the effect of daily renewal of this atmosphere. Comparison of these results with those of the 3.0-percent-oxygen atmosphere without carbon dioxide (table 1) shows (1) that the presence of carbon dioxide in this atmosphere is of no significance in the preservation, or loss, of carotene; (2) that a daily renewal of the storage atmosphere results in a progressive and decidedly greater loss of carotene (32.5 percent), almost exactly the loss occurring in the same length of time (56 days) in air.

When the percentages of carotene losses in 17 days are plotted against the percentages of oxygen in the storage atmosphere (fig. 5), it is seen that, with one exception (the 3.0 percent oxygen) the points

lie on, or are very close to, a straight line passing through the origin. It may therefore be concluded that at least the initial rates of carotene destruction are very nearly proportional to the percentages of oxygen in the storage gases.

The apparent gains in carotene content in nitrogen (containing 0.3 percent oxygen) after 17 days, and the similar trend in atmospheres containing 1.6 and 3.0 percent oxygen, cannot be explained as due solely to the limits of experimental accuracy of the determinations. In all the analyses in which the carotene content of the sample in each of two tubes stored for the same length of time in the same atmosphere was determined in triplicate, the average difference in carotene between the two tubes was less than 4 parts per million, the maximum being 5 parts per million (in 2 out of 9 cases). The average of the maximum differences in individual determinations of carotene made in triplicate for each tube was 11 parts per million. This difference can be accounted for by variations in the triplicate samples taken from the contents of the tube, but error from this cause would be expected to disappear largely in the average of the three determinations representing 60 to 80 percent of the total contents of the tube.

A similar apparent increase in the carotene content was reported by Wilder and Bethke [8] for samples of alfalfa meal in nitrogen-packed, metal containers stored at room temperature (between 21° and 23° C.) and at 37° C. and likewise for samples packed in vacuo, in similar containers, and stored at the same temperatures.

The writers do not believe that there was an actual increase in carotene in atmospheres of low oxygen content. On the other hand, an application of calculated probable error of the analytical determinations of carotene to each point of the curves (fig. 3) is not sufficient to eliminate the general upward trend in the curves. The cause of the apparent increase in carotene during storage in atmospheres of low oxygen content cannot be explained. The formation of coloring material that behaves like carotene in the physical method used for carotene estimation can be assumed to be the cause; however, such formation of color might also take place in atmospheres of higher oxygen content but be overlooked because of the destruction of more color by oxidation of carotene. This would explain why the upward trend in the curves appears earlier in cases of storage in low concentrations of oxygen. Whatever may be the explanation, the results of the carotene determinations for alfalfa stored in atmospheres of different oxygen content would still remain relatively comparable with each other, and would unquestionably indicate the least loss of carotene from storage in the atmosphere of lowest oxygen content.

Carotene losses were greatly reduced in the atmospheres of low oxygen content in the closed containers. The percentage loss of carotene resulting from the daily renewal of a 3-percent-oxygen atmosphere for 56 days was as great as that in air in the closed containers for the same number of days. From these facts the conclusion was reached that storage in the closed containers was accompanied by a reduction in oxygen concentration in the initial stages of storage through oxidation of constituents of the alfalfa other than carotene, and in consequence loss of carotene was retarded or prevented entirely.

[8] See footnote 4, p. 362.

The correctness of this conclusion was confirmed by the following experiment:

A mixture of air and nitrogen containing approximately 3 percent of oxygen was prepared. Two large U tubes of 410-cc. capacity provided with vacuum-tight stopcocks were used as containers for the alfalfa; 85.8 gm. from the same lot of alfalfa as that previously used was weighed into each of the U tubes, this weight being that required for the same proportion of meal to capacity of container as in the previous experiments. In addition to these samples four other samples in small U tubes were provided for carotene and moisture determinations. The tubes were conditioned for storage by the passage of 8.7 liters of the gas in 6⅓ hours at room temperature, and following this operation the large tubes were placed in the oven at 40° C. and conditioned for humidity by the passage of 6 liters of the gas in 5$\frac{5}{12}$ hours, the gas first having passed through a 20-percent solution of sodium chloride at 26.7° in the humidifier shown in figure 1. The gas emerging from the tubes at the end of this operation was found to contain 3.25 percent of oxygen, and this value for oxygen was recorded as that of the storage atmosphere. The tubes were then closed and left in the oven for 28 days. At the end of 28 days they were removed from storage, and samples of the gas were collected under reduced pressure from the tubes and analyzed. Carotene and moisture in the alfalfa after storage were also determined.

In both tubes the oxygen had been completely consumed. The carotene loss was only 11 parts per million, or 5.6 percent, which is far too small to account for the oxygen consumption. This loss is in remarkably close agreement with the losses in 28 days in approximately the same concentration of oxygen recorded in tables 1 and 2. The carbon dioxide produced was found to be in excess of the oxygen consumption, being 5.8 and 5.7 percent in the two tubes.

SUMMARY AND CONCLUSIONS

The storage of alfalfa meal at 40° C. in closed containers in contact with atmospheres of low oxygen content resulted in a high degree of carotene conservation. The presence of carbon dioxide in these atmospheres had little effect.

Conservation of carotene was due in large measure to a reduction in the original oxygen concentration by oxidation of constituents of the alfalfa other than carotene in the early stages of storage.

A daily renewal of the storage atmosphere containing oxygen was accompanied by a great increase in carotene loss.

It therefore follows that the most effective conservation of carotene in alfalfa commercially by means of inert gas would be accomplished by the highest possible exclusion of air throughout the entire storage period. The extent to which oxygen can be excluded from the storage atmosphere economically in commercial practice remains to be investigated. It would appear from a comparison of the curves in figure 4 that the permissible oxygen content of the storage atmosphere at the beginning will be somewhat less than 3 percent.

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